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Title:
A study of the activity and species richness of British bats and their insect prey on organic and conventional farms using acoustic survey methods

A study of the activity and species richness of British bats and their insect prey on organic and conventional farms using acoustic survey methods

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ABSTRACT

In order to evaluate the impact of agrochemicals on British bat populations, bat activity was monitored and species richness determined using a reliable acoustic technique within different habitats (pasture, woodland, arable and water) on matched pairs of organic and conventional farms. Insect abundance and species richness were studied at the same time. The reliability of different methods of frequency reduction and transformation techniques used to study the echolocation signals generated by bats was first investigated.

The main findings and conclusions were as follows.

- Frequency divided calls transformed with zero-crossing analysis resulted in inaccurate call descriptions, which sometimes lead to the misclassification of certain bat species. With their high information content, time expanded calls transformed using Fast Fourier Transformation resulted in more accurate call descriptions and classifications.
- For the method of species identification, artificial neural networks achieved higher correct classification rates than discriminant function analysis.
- The acoustic method employed in the farm study (direct sampling), was considered to be the most suitable method of echolocation recording for the purpose of this study, as it enables continuous sampling and results in calls with the same or higher information content as time expanded calls; species were determined using artificial neural networks.
- Higher overall bat activity was found on organic farms than on conventional farms. Within habitats bat activity was higher over water bodies in organic farms than over water bodies in conventional farms.
- Higher overall foraging activity was found on organic farms than on conventional farms. Species composition was different on the two farm types – total *Myotis* activity, and the activity of *M. daubentonii* and *M. brandtii*, were higher on organic farms.
- Total insect abundance and species richness was higher on organic farms than on conventional farms. Within habitats insect abundance was higher in organic pastural and over water habitats than in the conventional habitats.

- Insect dry mass was higher on organic farms overall, and within pastural and woodland habitats than on the same habitats on conventional farms.
- Of 18 key insect families identified as important to bat diet, five had significantly higher numbers of insects belonging to those families on organic farms than on conventional farms.
- These data on paired farms support the view that increased agrochemical use through agricultural intensification has had a detrimental effect on British bat populations through the reduction in prey availability and, possibly, alteration of important habitats.

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Finally the biggest thank you to my family. To my brother and sister, you helped me in many ways, whether it was just to be able to moan down the phone, or for fun times away from Bristol. To my parents who helped me financially, for their belief in me, their encouragement, constant support, and advice; this thesis is dedicated to them.

DECLARATION

I declare that the work in this dissertation was carried out in accordance with the regulations of the University of Bristol.

Stuart Parsons designed and trained the artificial neural network program used in Chapter 3, and provided the neural network program used in Chapter 4.

Other than this, I declare that all work in this thesis is my own, and no part of the thesis has been submitted for any other degree. Any views expressed within the dissertation are those of the author and do not represent the views of the University of Bristol. This work has not been presented to any other University for examination, either in the United Kingdom or overseas.

Signed:.....

Date:17/1/04

LIST OF ABBREVIATIONS

ANN	- Artificial neural networks
ANOVA	- Analysis of variance
BAPs	- Biodiversity action plans
BC	- Before Christ
cm	- Centimetres
CF	- Constant frequency
C-freq	- Central frequency
DAT	- Digital audio tape
DDE	- Dichlorodiphenyldichloroethylene
DDT	- Dichlorodiphenyltrichloroethane
DFA	- Discriminant function analysis
Dur	- Duration
EC	- European community
FD	- Frequency divided
FD/ZCA	- Frequency divided calls transformed using ZCA
FD/FFT	- Frequency divided calls transformed using FFT
F-end	- End frequency
FFT	- Fast Fourier transformation
FM	- Frequency modulated
FmaxE	- Frequency of maximum energy
F-start	- Start frequency
GIS	- Geographical information system
GM	- Genetically modified
m	- Metres
SEM	- Standard error of the mean
TE	- Time expanded
TE/FFT	- Time expanded calls transformed using FFT
TE/ZCA	- Time expanded calls transformed using ZCA
UK	- United Kingdom
USA	- United States of America
ZCA	- Zero crossing analysis

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CHAPTER 1

INTRODUCTION

1.1 Population declines

1.1.1. Global biodiversity declines

The continued loss of biodiversity from this planet as a result of human activities is a stark reminder that, although conservation biologists are trying hard to alleviate the rate of population declines and species extinction, more information is needed about the underlying mechanisms of decline, and how the already well known causes of decline affect particular species. These data are an essential prerequisite to planning future management policy.

Recent estimates of impending rates of species loss are between three and five orders of magnitude higher than background extinctions levels (May *et al.* 1995; Pimm *et al.* 1995). Anthropogenic factors, such as land development, over-exploitation, species translocation and introductions, and pollution, are the primary causes of extinctions (Mace *et al.* 1998). The decline of a number of species and possible causes of such declines has been well documented in the literature over the years. Taxa for which population declines have been documented include amphibians (Harvell *et al.* 2002; Collins & Storfer 2003; Funk & Mills 2003), primates (Meijaard & Nijman 2000; Harcourt *et al.* 2002; Waltert *et al.* 2002), birds (Chamberlain *et al.* 1999; Ratcliff & Crowe 2001; Fuller *et al.* 2002; Murphy 2003) and aquatic animals (Xie & Chen 1999; Carr *et al.* 2002).

Habitat fragmentation as a result of changes in land use is one of the major driving forces of population declines in both temperate and tropical regions. For example, forest fragmentation results in population declines and extinction for many

forest vertebrates (Funk & Mills 2003). Agriculture is the dominant land use in the world covering over one third of the planet's exploitable surface (Dobson 1998). Throughout the world key natural habitats, whether forests or savannah, are being converted to agricultural land (Dobson 1998) or being farmed increasingly intensively to increase outputs. The widespread detrimental effects of agriculture on biodiversity are well documented, and the severity of the situation is becoming increasingly evident in terms of species declines.

1.1.2. Farmland species declines

As the major land use, agriculture affects wildlife populations on a national and international scale (Fuller *et al.* 1995). Since population monitoring began in the 1960s, farmland bird populations have shown declines in Northern Europe. Two well known case studies are on the skylark *Alauda arvensis* and the grey partridge *Perdix perdix* (Sotherton, 1998) although many other bird species have also shown declines (Siriwardena *et al.* 1998; Ormerod & Watkins 2000; Ambrosini *et al.* 2002; Benton *et al.* 2002). The declines of many of these species occurred at a time when rapid changes in farmland management were taking place (Chamberlain *et al.* 1999). Of the 28 farmland bird species studied in the UK, 24 have shown a contraction in range between 1970 and 1990 (Fuller *et al.* 1995). The loss of traditional rotations in farm management has led to a decrease in habitat available to birds, insects and other wildlife on farms. Farmland species are heavily reliant on farmland habitats for breeding sites and foraging areas. This makes the study of agro-ecosystems important for wildlife conservation.

Other taxa that have shown population declines on farmland include spiders and several insect orders including ground beetles (Tischler, 1980; Aebischer, 1991; Feber *et al.* 1997; Chamberlain *et al.* 1999). Worryingly for farmers, the widespread use of pesticides has caused the unintentional destruction of beneficial insect predators of

pests, thereby increasing the population of many species of agricultural pests and the associated risk of disease (Pimentel & Greiner 1997; Wilson & Tisdell 2001). In a recent government report on the pros and cons of genetically modified (GM) crops (GM Science Review 2003), the greatest perceived risk from GM crops is the impact they will have on weed communities and insect 'pests' which are valuable prey for farmland predators such as bats and various birds.

Although there is a growing amount of evidence implicating agricultural intensification in biodiversity declines, the underlying functional mechanisms (for example effects on food webs) driving these declines remain unclear. An effective way of monitoring environmental change and biodiversity declines is through the use of bioindicator species.

1.2 Bioindicators

1.2.1. Qualities of bioindicators

Bioindicators can be used cross-sectionally to assess the status of an ecosystem or its components, or longitudinally in a monitoring framework (Burger *et al.* 2001). Studying bioindicators is useful where an extensive survey of multiple species within an ecosystem is unfeasible. One of the primary uses of indicator taxa is for monitoring environmental change (Mace *et al.* 1998). The ideal indicator taxon is hard to find when trying to answer an ecological question. It must posses an ensemble of traits rarely found in one taxon (Mace *et al.* 1998).

Bioindicators should be geographically widespread, be responsive to stresses and enable changes in population status to be measured. These changes must be attributable to a cause and be important to the well-being of the organism and important to other components of the ecosystem (Burger *et al.* 2001). Other characteristics include

being restricted to specific habitats and highly susceptible to pollutants (Bright & Morris 2000). Bioindicator-based studies can help in the process of amelioration and remediation of the rural landscape, such as to assess the benefits of reducing agricultural pesticide use (Pimentel 1997; Paoletti 1999a).

A large variety of organisms have been classed as bioindicators. These include waterbirds (Custer *et al.* 1991; Fox 1994), some invertebrates (Paoletti 1999b; Rainio & Niemela 2003) and some mammals (Scholey 1993; Fox 1994). When it comes to bioaccumulation of pollutants, indicators are often familiar species that are high up on the food chain (Palmers 1993; Fox 1994). The sparrow hawk *Accipiter nisus* and otter *Lutra lutra*, both protected and presently recovering following pesticide-induced declines, are two such examples (Newton & Wyllie 1992; Strachan & Jefferies 1996). Indicator species are also normally currently rare or declining.

1.2.2. Why study bats?- bats as bioindicators

Bats are the second most specious order of mammals and are distributed world-wide (Altringham, 1996). Their varied diet and ability to fly has almost certainly aided in their global distribution. European bats belong to the suborder Microchiroptera, and are nocturnal insectivores. In the UK, bats are the most important contributors to terrestrial mammalian species biodiversity (Mickleburgh *et al.* 2001).

Bats possess ecological and life history traits that make them a key group for ecological studies, not only as a group in their own right, but also as a model group to investigate and answer other broader ecological questions. Bright & Morris (2000) suggested that a good indicator group would be the 'sequential specialists', whose fitness depends on a chain of highly specific and mostly ephemeral resources. Bats such as *Rhinolophus ferrumequinum* fit into this category, being critically dependent on

seasonal insect availability within specific habitats, which must be close to roost sites (Bright & Morris 2000).

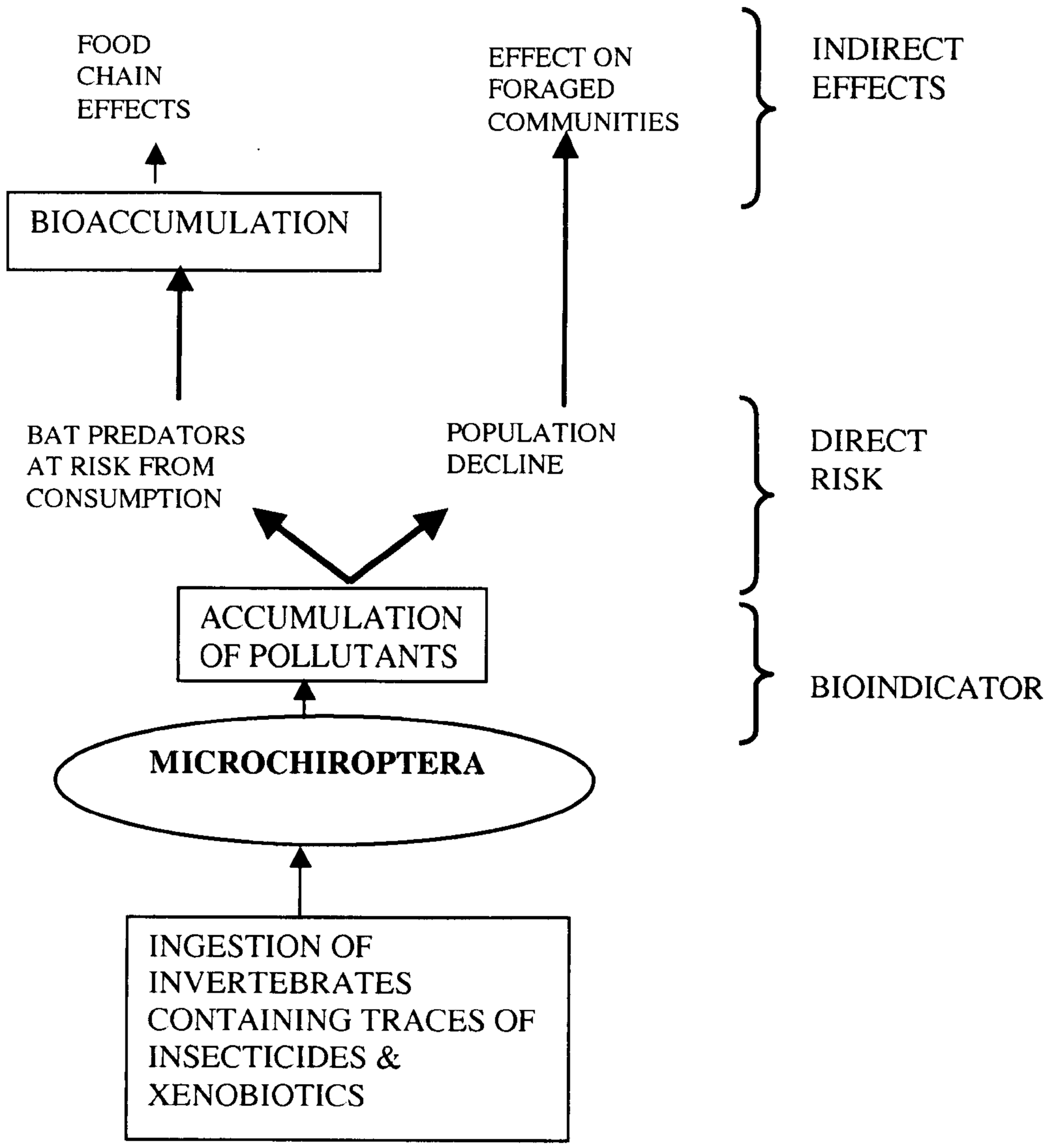
Bats are top in the food chain; being insectivorous they form part of a food chain common to a number of animals and are sensitive to pollutants accumulated in their prey e.g. insecticide traces from farming practices (Fig. 1.1). There is limited data to suggest bat populations are resource limited (Bonaccorso 1979; Findley 1993) and they are highly sensitive to any environmental changes that may alter the abundance of their food supply. These changes include habitat destruction, habitat modification, urbanisation and forms of pollution. There have been many papers published on the detection of organochlorine insecticide and pesticide residues in birds (Gervais *et al.* 2000; Smith & Bouwman 2000; Minh *et al.* 2002), and field and laboratory data on lethal brain residues of dieldrin, DDT and DDE suggest that bats are similar to birds in their sensitivity to organochlorines (Clark 1981). Bats have been found dead with high levels of DDT in their tissues and although DDT has now been banned in the UK. More recently lindane used for as a wood preservative to treat roof timbers was shown to significantly effect the energetics of pipistrelle bats (Swanepoel *et al.* 1999) This susceptibility to the bio-accumulation of toxic compounds illustrates the sensitivity of these creatures to environmental change (Jefferies 1972; Timbrell 1995).

In 1995 bats in southern USA still contained high levels of DDT (Timbrell 1995), even though it was no longer in use. This was probably because there was sufficient residual DDT in the environment for it to appear in food chains. Also, annual cycles of fat depletion increase contaminant concentrations in hibernating bats. The transfer of chlorinated pesticides from mother to juvenile during lactation means that toxicity may occur following nursing or later when the pesticides are mobilised to the brain as fat reserves are depleted when the young begin to fly (Clark 1981). This

delayed toxicity effect could also occur when fat reserves are depleted late in hibernation (Clark 1981).

Bats are ecologically linked to specific features of the landscape and use linear features (e.g. hedgerows or woodland edges) as flight paths connecting foraging sites (Verboom & Huitema 1997). Most species of animal can react to environmental change and adopt new patterns of behaviour to cope with the change (Paoletti & Bressan 1996; Paoletti 1999a).

Fig. 1.1 Schematic of bioindicator properties of insectivorous bats.



Due to their longevity and slow reproductive cycle, bats have a very limited ability to recover from population declines, and because of their roost loyalty, bats often cannot disperse to avoid declines in habitat quality. These ecological properties mean that bats may be unable to react quickly and adapt to environmental changes and are less likely to adopt new patterns of behaviour.

The fact that many bat species are declining across Europe (Mitchell-Jones 1995; Hutson *et al.* 2001) and are consequently protected by both European law and national legislation adds weight to the evidence that as a group of animals, bats are important indicators of change, and highlights the need for quantified data on the effects of agricultural intensification on this group.

1.3 The health of intensively managed ecosystems

1.3.1. A brief history of intensive agriculture

Agriculture was the first step in aiding human expansion. By 4000 BC the wildwood that covered much of the UK began to be cleared for agriculture, which marked the beginning of a phase of anthropogenous enrichment and differentiation of vegetation (Pott & Hüppe 1991; Elsen 2000; Rackham 2000). The economic adaptations of the early post-glacial period were replaced from the 7th to the 5th millennia BC by an economy based on cereal cultivation and the husbandry of a restricted range of species (Champion *et al.* 1984). The initial intensification of agriculture took the form of rotations and land reclamation, e.g. the draining of fenlands in eastern England.

Modern agricultural intensification is defined as increased production of agricultural commodities per unit area (Donald, Green & Heath 2001). The synthesis of chemical fertilisers and the onset of the industrial revolution from the 1800s onwards paved the way for modern intensive agriculture aided by increased mechanisation. The

intensification of agriculture brought about high output yields through the increased use of synthetic chemical fertilisers and pesticides. These high yields made it possible for the ever-increasing human population to be fed from relatively smaller land areas. Nevertheless there has been destruction of habitats within and immediately surrounding intensively managed farmland (O'Connor & Shrub 1986; Altieri 1999; Mickelburgh *et al.* 2002), especially since the 1940s (Table 1.1).

Table 1.1 Percent habitat loss in UK since the 1940s. Data from the Countryside Agency Research notes (2002).

Habitat type	**% loss since 1940s**
Unimproved neutral grassland	97
Wetland	90
Broadleaved woodland	50
Hedgerows	45
Herb-rich meadow	95
Ponds and ditches	30
Heathland	75

Intensive farming practices involve the abundant use of synthetic fertilisers and pesticides and results in the fragmentation of wildlife habitats (Burgess & Sharpe 1981), the degradation of soils and the pollution of groundwater (Berka *et al.* 2001; Honisch *et al.* 2002).

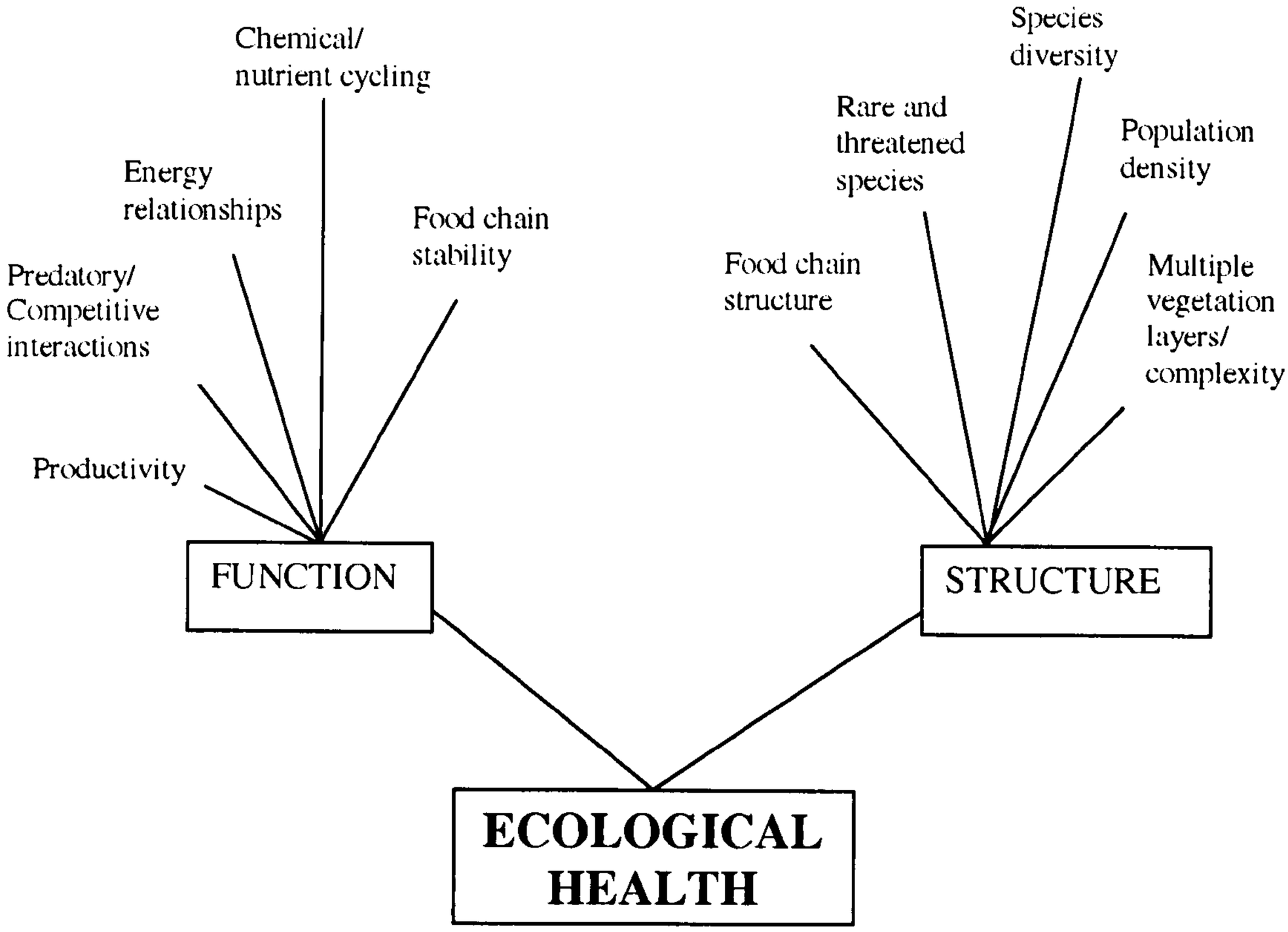
1.3.2. Simplification of the landscape

Ecological health can be viewed strictly in terms of the maintenance of the functional characteristics (including predatory or competitive interactions, energetics) and structure (e.g. species diversity, population density) of ecosystems (Fig. 1.2) (Burger *et al.* 2001).

Some land management decisions made by intensive farmers to maximise output result in the landscape becoming homogeneous and simplified (Sotherton 1998). The

loss of traditional rotations due to the intensification process has led to a decrease in the diversity of habitat available for wildlife on farms. The removal of non-crop habitats has also contributed to the decline in habitat diversity e.g. hedgerows, ponds, copses (small areas of woodland) and field margins (Robinson & Sutherland 2002). Removal of boundaries to make fields larger (Chapman & Sheail 1994; Sotherton 1998) not only reduces insects through loss of vegetation, but loss of hedgerows also removes shelter for both insects and those animals that utilise linear features e.g. bats.

Fig. 1.2 Characteristics of ecological health (Adapted from Burger *et al.* 2001).



On farmland, bats fly along windbreaks such as hedgerows, which attract and provide shelter for flying insects. These features serve as flight corridors as well as foraging areas (Verboom 1997). Connectivity in landscapes, which is reduced by intensive management, is important (Walsh & Harris 1996b).

Insect activity is highly dependent on environmental factors such as wind speed and temperature. The removal of woodlands and hedgerows increases mean surface wind speeds, which could affect insect activity and therefore food resources for insectivorous animals. In Britain it is estimated that between one quarter and one third of hedgerows have been removed since 1945 (Watt & Buckley 1994). A recent review of the literature on farmland biodiversity declines showed that habitat heterogeneity was associated with higher biodiversity in the farmland landscape, and that a loss in habitat heterogeneity at multiple spatial scales is a major cause of biodiversity declines in farmland (Benton *et al.* 2003).

1.3.3. Landscape changes and implications for wildlife

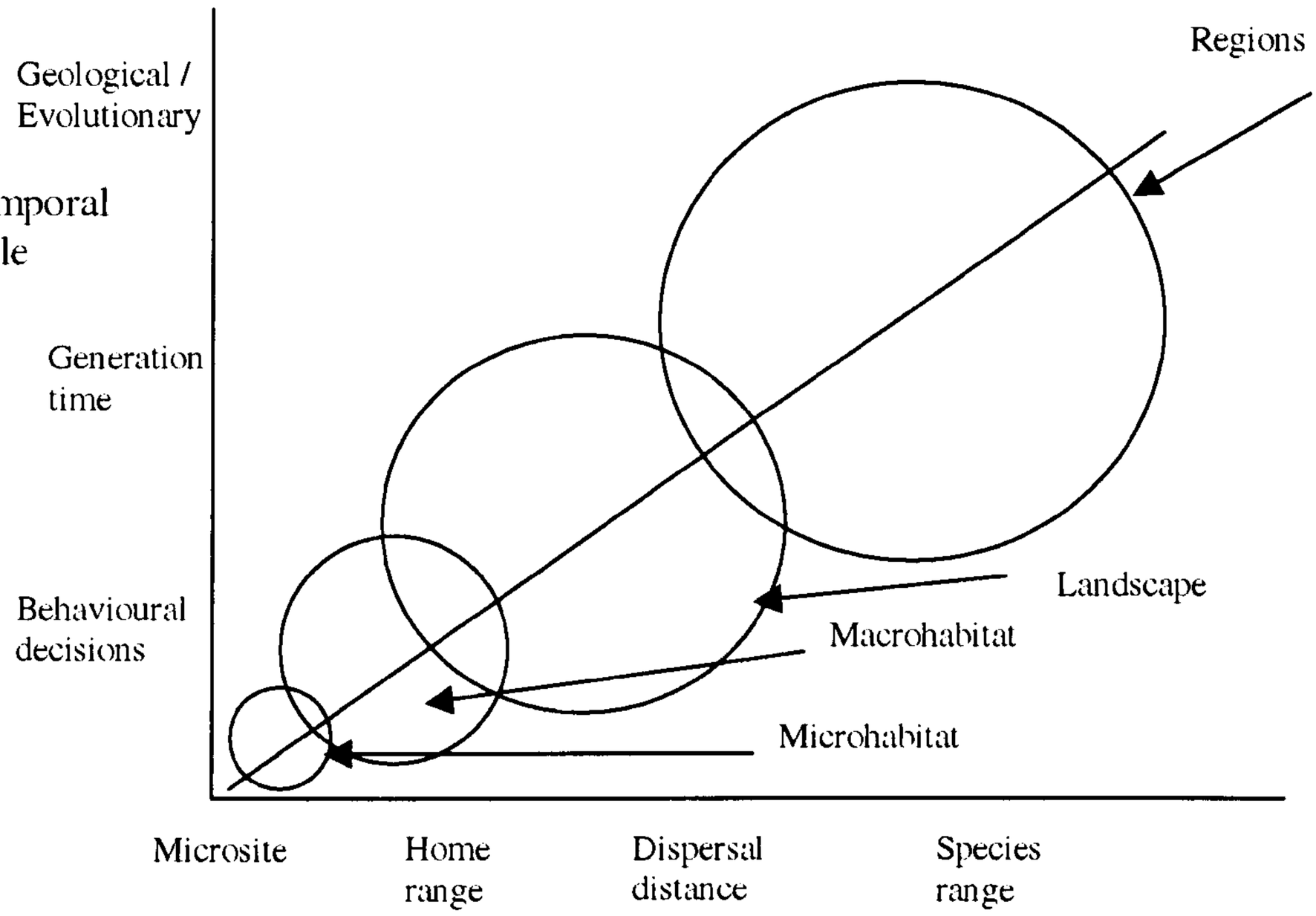
Understanding the effect of landscape dynamics on mammal distributions is essential if populations are to be managed effectively (Gough 2000). Landscapes are heterogeneous areas comprised of patches of habitat in a matrix or mosaic (Turner 1989), each patch being an area that differs from its surroundings. The way in which a species interacts with the landscape varies depending on the spatial and temporal scale at which it operates as well as the landscape itself. Connectivity, for example, depends not only on the distance between patches, but also on the ability of the animal to move between them (With & Crist 1995; O'Neil *et al.* 1998; Gough 2000). The energetic cost of travelling between multiple patches can become unsustainable if the habitat becomes too fragmented (Hinsley 2000).

A habitat is not static but is continuously changing as a result of natural or anthropogenic disturbances operating on many spatial scales. The choice of a foraging site (microhabitat) by a bird, for example, may change every few minutes based on prey availability, temperature, wind speed and presence of predators or competitors. The

regional range of a species is generally stable over long time periods (Fig. 1.3). Habitat alterations across the range of a species may influence the suitability of that habitat at scales beyond those at which an individual can respond (George *et al.* 2001). For mobile species, and in particular species that are loyal to roost sites and foraging flight paths such as bats, or species that use ephemeral habitats, the temporal duration of suitable habitat may be more important than the distance between patches.

Studies have reported that bat communities are divided into habitat type specialists, e.g. gap specialists (Crome & Richards, 1988; Hayes, 2000). In one study (Sherwin *et al.* 2000), bats were divided into guilds based on foraging strategy and frequency of calls. The guilds were gleaners (a foraging strategy involving gleaning prey from stationary objects), aerial hawkers (a foraging strategy involving prey capture on the wing), mixed strategists and unknown. Results showed that guilds were not randomly distributed among habitats. Differential habitat use by bats suggests that landscape level changes through habitat perturbation could cause habitat use changes in local bat communities (Sherwin *et al.* 2000). Determining which agent or agents push a species towards extinction is pivotal to conserving it (Caughley & Gunn 1996).

Fig. 1.3 Relationship between temporal and spatial scales and levels of habitat selection. Processes operating at small spatial scales occur over a short time, and those at larger scales take place over long periods (George *et al.* 2001).

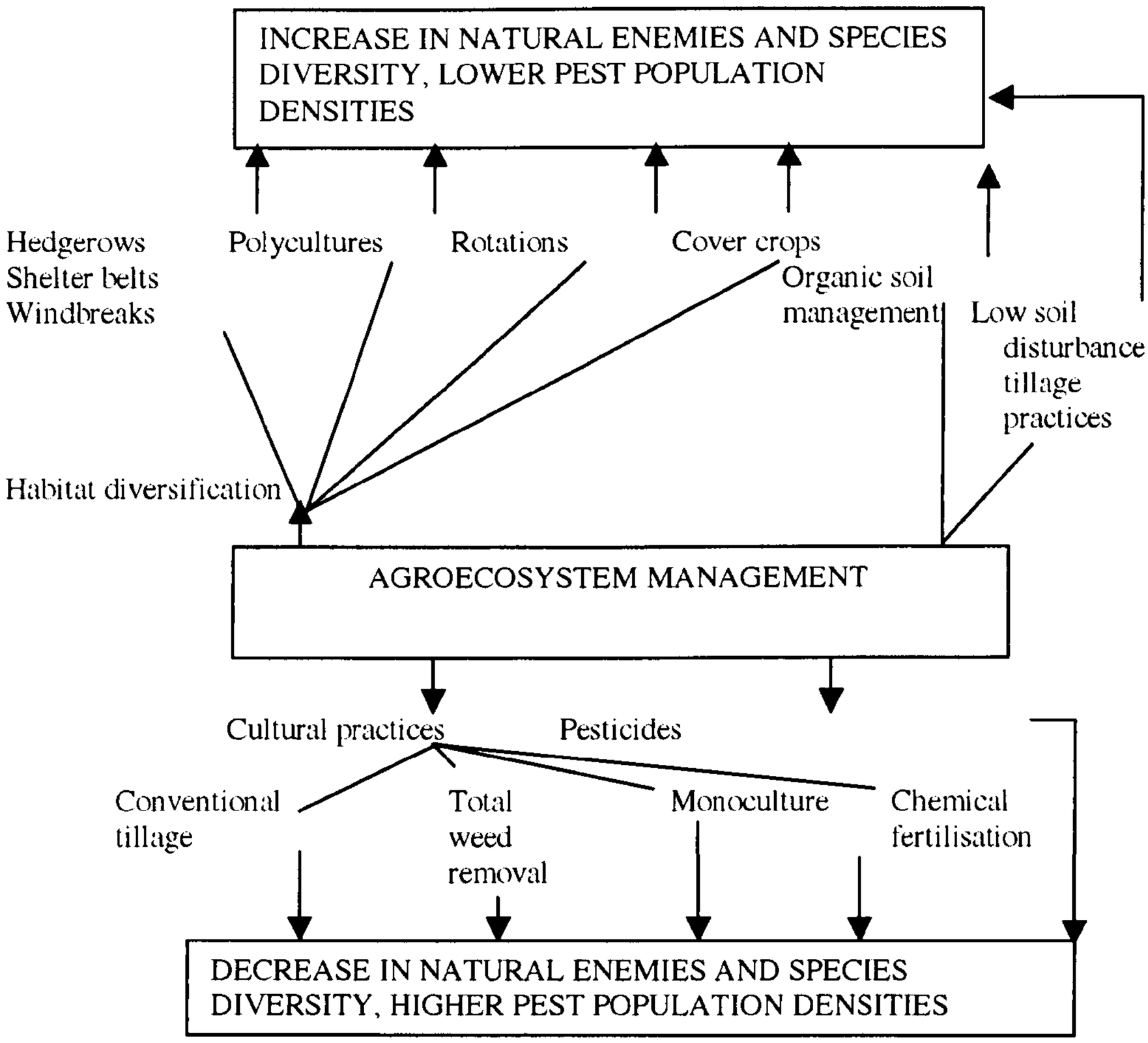


1.3.4. Organic farming – a model system

In agricultural systems, biodiversity provides many ecosystem services including recycling of nutrients, regulation of local hydrological processes, detoxification of noxious chemicals and regulation of undesirable organisms (Fig. 1.4) (Altieri 1994). When these natural services are lost due to biological simplification, the economic and environmental costs can be significant (Altieri 1999).

Modern agricultural systems have become very productive, but only by being highly dependent on external inputs. Organic farming uses some of the same techniques and holds the same philosophy as the traditional farming systems discussed earlier. This form of farming has existed since the intensification process took hold in the 1940s but has been given a new lease of life in the last decade.

Fig. 1.4 The effects of agroecosystem management and associated cultural practices on the biodiversity of natural enemies and the abundance of insect pests (Altieri 1999).



Due to growing concerns about the environmental consequences of modern intensive systems, animal welfare and recent food scares, organic farms are on the increase. More conventional farms convert to organic management every year. Organic farming aims to create an integrated, humane, environmentally and economically sustainable system of agriculture (Lampkin 1998). Organic farming is different from other forms of sustainable agriculture in having a set of legal and voluntary standards and a rigorous certification process associated with it. Figures published by the Soil Association, the biggest national organic certifying body in the UK, state that in 2001 organic farming covered 552,500 ha of land, 3.2% of the total farmed area in the UK. The UK organic market grew by 33% in the year 2000/2001. It is estimated that at the current rate of

growth 10% of Western European agriculture will be organic in 2005, and 30% by 2010 (The Soil Association 1999).

Organic farming systems used today combine modern and traditional farming concepts to enable the farmer to work with the environment with benefits to livestock care and wildlife. Mixed farming is normal on organic farms, and this provides a range of wildlife habitats across the farm area. The majority of organic farms have both crops and livestock, and use a rotation system. Rotations involve the use of grass or clover leys and are a means of achieving pest and weed control. Pest control is achieved through the maintenance of natural predators such as spiders and birds. The maintenance and management of trees, hedges and field margins is an essential part of organic farming and these are protected under organic standards. The avoidance of synthetic agrochemicals is a key feature of the organic system and means that it is an ideal model system to investigate the effects of intensification on biodiversity.

Twenty-three studies carried out in the 13 years leading up to the year 2000 were undertaken in Europe to investigate the comparative biodiversity benefits of organic and conventional farming, the majority of which suggest lower biodiversity on conventional farms than on organic farms (Soil Association Report 2000). In a study of invertebrate and weed seed food-sources for birds on organic and conventional farming systems (Brooks *et al.* 1995), significantly higher numbers of common species of carabid beetle, earthworms and dipteran larvae were found in organic fields than in conventional fields. In the case of insects, the general reduction in plant diversity in hedgerow understories and grasslands has reduced the range and abundance of a number of food sources for many species (Feber *et al.* 1997). These authors used a paired farm approach involving organic and conventional farms to test the hypothesis that different farming systems support different levels of pest and non-pest butterflies. Their results showed more

butterflies on organic farms than on conventional farms (Feber *et al.* 1997). Other invertebrates like bees, butterflies and spiders have been found to have 50-700% more species per unit surface on organic farms (Mansvelt 1998). In a study looking at landscape features on organic and conventional farms in the Netherlands, Germany and Sweden, diversity in terms of land-use and crops was greater in organic farms than in conventionl farms. Vertical and horizontal coherence were analysed and found to be greater in organic farms (Mansvelt 1998).

However, before the activity of a group of animals can be monitored, an accurate technique of species identification is required. Bats pose unique problems when it comes to species identification, which is an essential factor in assessing population status.

1.4 Species identification of bats

1.4.1. Identification methods for bats

When studying the ecology of communities of animals, a number of variables are quantified and measured. These include abundance or activity levels, habitat use, and information on the prey base. Accurate information on species diversity is crucial to derive species-specific data. The identification of bat species is required for the assessment of foraging habitats and, subsequently, habitat conservation (Vaughan *et al.* 1997).

Being nocturnal fast-flying animals, some of the usual methods of identification, such as field signs, are inapplicable. Bats in flight are difficult to identify by visual techniques (Jones *et al.* 2000). Bats may be identified in the hand on the basis of morphological criteria. The bats are trapped using mist nets or harp traps set up at entrances to roost sites or caves, identified in the hand and subsequently released.

Netting is ideal for catching in enclosed spaces like roost entrances. However, netting is not practical for surveying a number of habitats in the open. Nets have to be manned and high-flying species are missed, leading to sampling bias. Some species of bat are difficult to tell apart by morphological criteria, especially cryptic species (Jones 1997; Jones & Barlow 2003). Acoustic analysis of the echolocation calls emitted by bats is a method of species identification employed in many field studies dealing with free flying bats. Monitoring echolocation calls through acoustic methods can determine patterns of distribution and habitat use (Fenton 1997).

1.4.2. Echolocation

Echolocation is a technique used by certain organisms to gain information about their environment by sending a probing stimulus. The strategy is most commonly used where darkness or turbidity limits vision (Dusenbery 1992). Of the animals that use echolocation to probe places where light is unavailable, microchiropteran bats are among the most adept (Wade 2000).

Echolocation involves the emission of pulses of sound by animals, and reception of the much fainter echoes that return from objects in their path, such as prey (Griffin 1958). For bats, a series of echolocation calls is defined as a bat pass (Fenton 1970). High-frequency echolocation is a good sensory system for detecting small nocturnal aerial insects, which make up the dominant part of the diet of microchiropteran bats, as the high frequencies return strong echoes from small targets (Jones 1999). Microchiropteran bats extract crucial information from the echoes of their signals; very detailed information about the surrounding habitat and potential targets are encoded in changes in the amplitude, frequency and time of the returning echo(es) in relation to the

outgoing signal. Processing takes place in the auditory cortex in order to determine direction, size, distance, shape, angle and velocity of a target (Neuweiler 1989).

A typical echolocation sequence from a foraging bat consists of search-phase calls to detect prey in the environment, approach-phase calls to locate and pursue prey and terminal-phase calls to determine final range (Parsons *et al.* 1997). The end portion of the acoustic sequence is commonly termed a 'feeding buzz', and signifies an attempt at prey capture (Griffin *et al.* 1960) (Fig. 1.5). It is defined as a characteristic increase in pulse repetition rate as the bat closes in on its prey, and can be used to distinguish between foraging and commuting bats. Search-phase calls are emitted more often than the other types of call making them ideal for the acoustic identification of species. Search-phase calls often also have species-specific characteristics (Ahlen 1981; O'Farrell *et al.* 1999). Bat echolocation calls consist of FM (frequency modulated) and CF (constant frequency) components. FM sweeps cover a wide frequency range in a short amount of time (Fig. 1.6). The returning echoes from FM calls encode precise information about target range and angle and are therefore useful for the localisation of objects (Schnitzler & Kalko 1998). Some bat species use long duration CF calls e.g. *Rhinolophus* species (Fig. 1.7). CF bats can detect fluttering targets amongst echo clutter as echoes from moving insect wings contain abrupt changes in frequency and intensity wherever the wing position is normal to the sound beam (Neuweiler 1989; Schintzler & Kalko 1998). The call structure of bats therefore reflect their foraging ecology (Neuweiler 1989); for example, species foraging in open habitats emit long calls at lower frequencies, which travel far to detect distant objects (Jones 1999). All microchiropterans probably use echolocation for orientation, but some species do not always use echolocation for the detection and localisation of prey (Jones 1999).

Fig. 1.5 Waveform of the end portion of the terminal phase of feeding behaviour, termed a feeding buzz. (*Pipistrellus pipistrellus*).

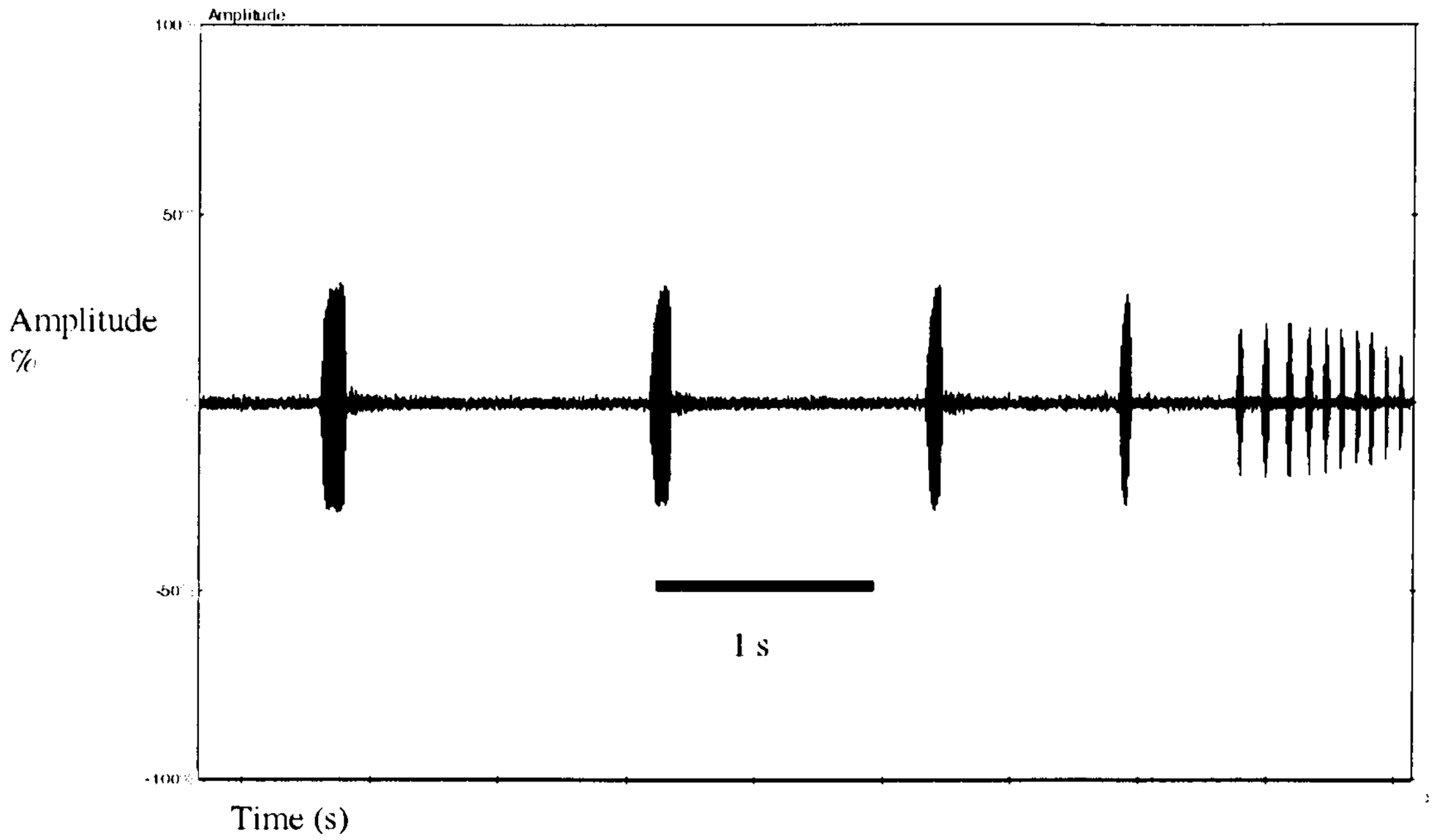


Fig. 1.6 Short frequency, frequency modulated sweep of a time expanded *Myotis daubentonii* echolocation call. Top chart displaying the waveform, bottom chart displaying the spectrogram.

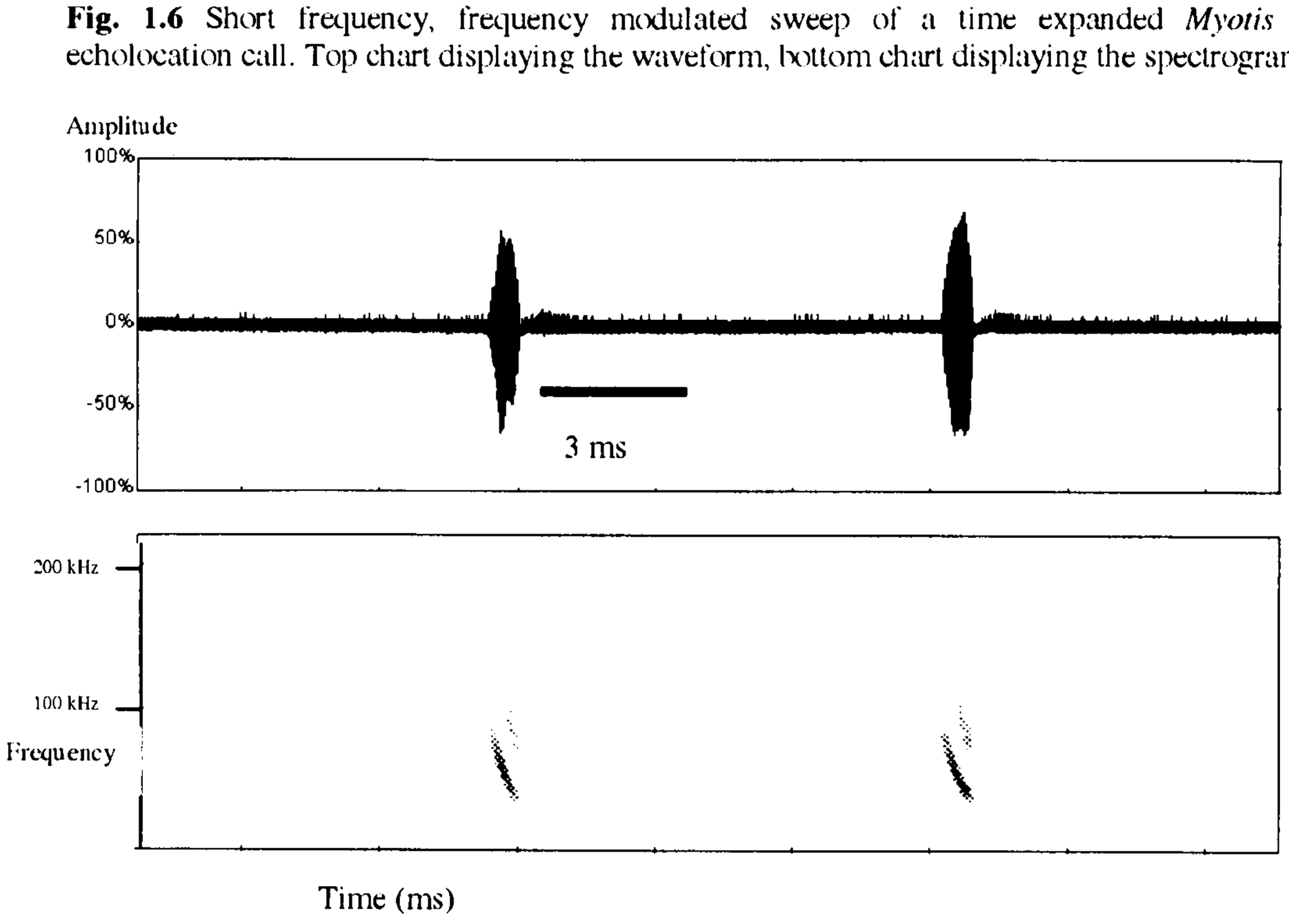
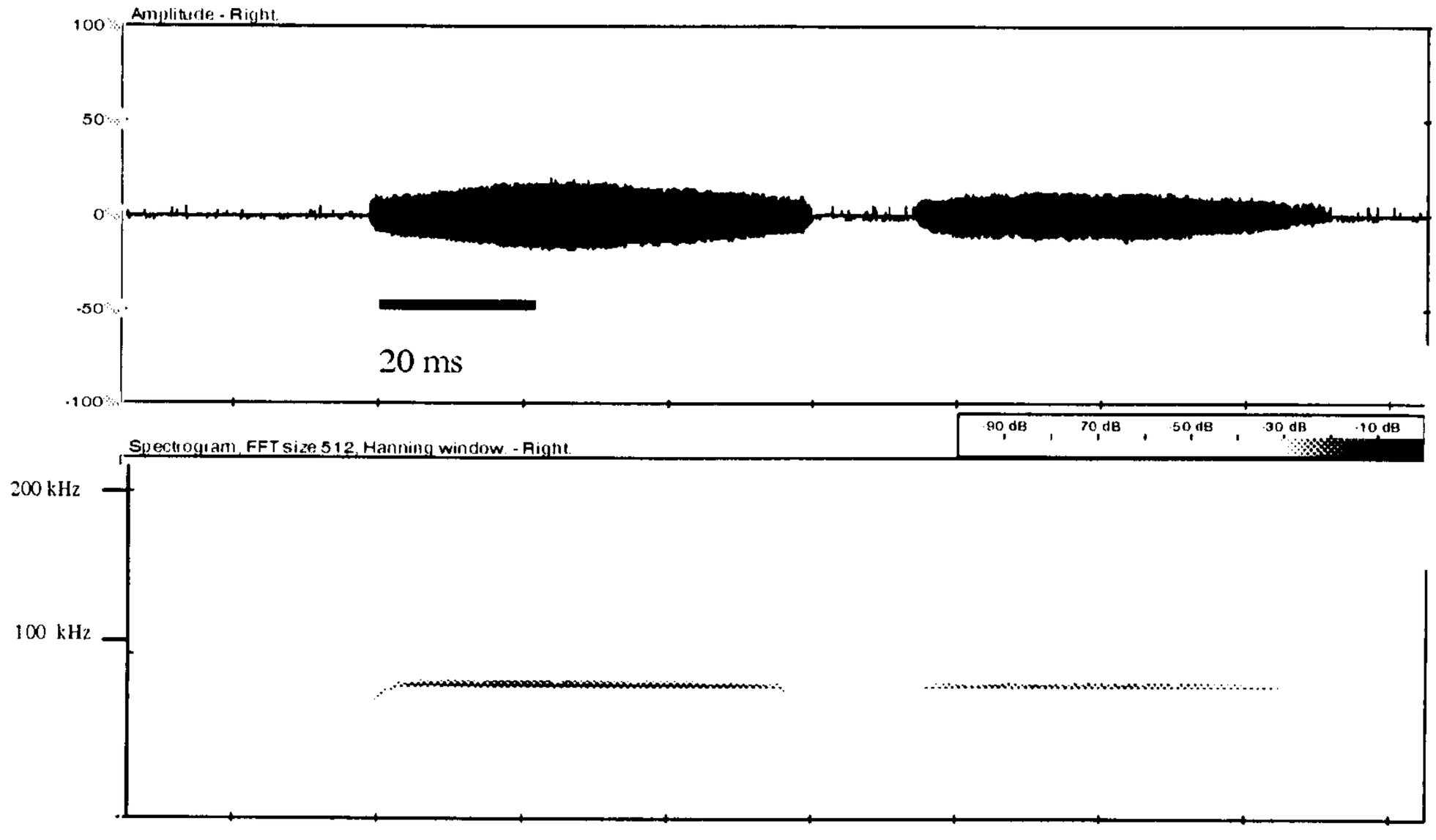


Fig. 1.7 Time expanded echolocation call of *Rhinolophus ferrumequinum*, showing the long duration constant frequency component. Top chart displaying the waveform, bottom chart displaying the spectrogram.



1.4.3. Variation in echolocation calls and problems with acoustic methods

Varying ecological conditions present different perceptual and echolocation challenges for bats. One of the main problems is the separation of target echoes from interfering factors, e.g. acoustic clutter, background noise, and sonar from other bats. To counteract these problems a wide range of call types have evolved in bats (Korine & Kalko 2001).

Echolocation calls vary interspecifically. They also vary intraspecifically due to the individual's age, sex and size (Buchler 1980; Jones *et al.* 1992), foraging strategy within certain habitats (Jacobs 1999; Jensen & Miller 1999) and acoustic clutter (Kalko & Schnitzler 1989; Rydell 1990; Obrist 1995). Variation within a species has also been attributed to geography (Barclay *et al.* 1987; Parsons 1997). Other factors have been linked with intraspecific variation in recorded echolocation calls such as Doppler-shift, atmospheric attenuation, and directionality of the bat detector and incoming emitted

signal (Obrist 1995; Parsons *et al.* 1997). Species-specific calls (Ahlen 1981; Fenton & Bell 1981) allow the identification of some species, a process constrained by intraspecific (feeding buzzes, search pulses, etc.) variation in calls (Rydell 1990; Obrist 1995). However, the fact that echolocation calls of some bat species are characteristic whereas the calls of others are very similar, species identification cannot always be made with certainty (Jones *et al.* 2000). In temperate regions the species that present the most problems in accurate discrimination belong to the genus *Myotis* due to similarities in call design. Identification of such species is possible through differences in start frequency, end frequency and bandwidth of the echolocation call (Parsons & Jones 2000; Jones *et al.* in press). The calls of different species are not equally detectable (Fenton & Bell 1981). It is important to note that acoustic survey methods are biased towards species of bat that emit high amplitude calls. Species such as *Plecotus auritus* emit very low amplitude calls, and some gleaners do not echolocate when locating prey. Low amplitude calls such as those emitted by the *Plecotus* species may be hard to detect when the bats are free flying in an open environment, leading to these species being under-represented in any acoustic survey. On the other hand, some bat species such as *Nyctalus* species produce high amplitude calls. Any detection method must be able to pick up on such variability. Indeed the equipment itself and the recording quality add to the variation.

The design and use of technologically advanced bat detectors has enabled field studies on bats to be undertaken with greater accuracy than previously. Sound analysis software and the techniques used to lower the high frequency calls of bats to present acoustic information to humans in an audible and visual format. As a result of improvements to the design and the portability of field equipment (Jones 1993; Waters & Jones 1995; Parsons & Obrist 2003) the analysis of echolocation calls from the field

is now widespread but the accuracy of species identification from such calls still depends on a number of variables. These include the sophistication of the equipment being used, the experience of the researcher and the knowledge of intraspecific variation in calls.

1.5 Aims of the study

The reported decline of many bat species drives the need for research into the main causes of these declines. Little is known about the detrimental effects of intensive farming on sequential specialists and bats are an ideal group to study this problem. Before we can investigate this, we need to establish an objective method of species identification.

One of the first tasks of this study will be to compare the efficiency of the two main techniques available for transforming the high frequency signal from a bat, to a lower frequency for analysis. Existing identification methods will then be tested in order to address the problem of inaccurate classifications. I then aim to investigate the impact of agricultural intensification on bat populations and their prey. To help address these aims I examine the following hypotheses:

1. Time expansion (TE) and frequency division (FD) give significantly different descriptions of echolocation calls.

In Chapter 2: *Acoustic species identification of UK bats (I)*, I determine the best system of species identification by testing if time expansion (TE) and frequency division (FD) give different descriptions of the same call, when recorded using the same equipment and analysed using similar methods. I show that the two techniques of frequency division and time expansion give significantly different descriptions of the same call. I

also show that the method of analysis used to measure call parameters gives significantly different measures of the same call.

2. Species can be accurately discriminated from FD calls using discriminant function analysis (DFA) and artificial neural networks (ANN).

In Chapter 3: *Acoustic species identification of UK bats (II)*, I investigate whether bat species can be as accurately identified from FD calls, using discriminant function analysis and artificial neural networks as from TE calls. I show that the discriminating capabilities of ANN and DFA on FD calls are less accurate than on TE calls.

3. Agricultural intensification has no effect on bat activity.

In Chapter 4: *Bat activity and species richness on organic and conventional farms: impact of agricultural intensification*, I investigate the extent to which the activity and diversity of bats found on farms differs with farm management, through the analysis of activity and foraging activity and the analysis of species richness within farmland habitats. Specific habitat aspects such as hedgerow height are also compared between farm types. I show that bat activity is significantly higher on organic farms than on conventional farms and that particular bat species are affected more severely by agricultural intensification than others. Organic farms are shown to contain higher hedgerows than on conventional farms, features that are used as flight paths by bats.

4. Nocturnal insect prey is equally abundant on organic and conventional farms.

In Chapter 5*: Nocturnal insect abundance and species richness on organic and conventional farms: implications of agricultural intensification for bat foraging*,

I evaluate the impact of agricultural intensification on the abundance and species richness of the insect base, with particular emphasis on key insect families important to bat diet. Insects were trapped using a variety of techniques within farmland habitats. I show that overall insect abundance and species richness is higher on organic farms. In addition I show that the abundance of insects belonging to the key insect families important for bat diet is higher on organic farms, highlighting the implications of agricultural intensification for bat foraging.

In Chapter 6*: Discussion*, I bring together the main findings from previous Chapters and discuss them in terms of conservation relevance in the UK. I then talk about the importance of the findings of this study in terms of global bat conservation and highlight points for further investigation.

CHAPTER 2

ACOUSTIC SPECIES IDENTIFICATION OF BRITISH BATS (I) – COMPARISONS OF CALL PARAMETERS DETERMINED BY FREQUENCY DIVISION AND TIME EXPANSION

2.1 Introduction

The accuracy with which bat species can be identified from their echolocation calls has been the subject of much debate (Barclay 1999; O'Farrell *et al.* 1999; Fenton *et al.* 2001). Bats are fast-flying nocturnal animals, species identification from echolocation calls is made difficult by the fact that the calls are ultrasonic, the extreme flexibility in the call design, technical limitations of the equipment used to detect and record calls, and the difficulty in obtaining true representations of original signals (Parsons *et al.* 2000).

For my study of differences in bat activity on organic versus conventional farms, it was crucial to be able to identify bat species as well as get an accurate indication of bat activity to collect data on species-specific habitat preferences. To quantify the differences between species of bat accurately, it was important first to determine the best method for recording echolocation calls and then test the main techniques for classifying bat species. There are two main systems of recording echolocation calls that utilise two different techniques for lowering the frequency of calls for subsequent analysis; time expansion (TE) and frequency division (FD). To view the frequency content of a call, it must also be transformed into the frequency-time domain, or the frequency-amplitude domain; using Fast Fourier Transformation (FFT) or zero crossing analysis (ZCA) (Parsons *et al.* 2000).

2.1.1. Problems with variations in call description - implications for survey work

Both FD and TE are used in bat research to describe calls from different species (Fenton & Bell 1981; Vaughan *et al.* 1997b; Parsons & Jones 2000), but there has been concern expressed over the degree of difference in call descriptions generated by the different equipment and techniques used. Time expansion bat detectors have been shown to be more sensitive and able to detect more calls per unit time and, presumably, at greater distances than frequency division detectors. The call descriptions resulting from both methods differ (Fenton *et al.* 2001). Fenton (2001) used different equipment, and so did not address whether the differences in call descriptions were due to the equipment used or were inherent in the frequency reduction (TE or FD) or transformation techniques (FFT or ZCA). Any differences generated by the two recording systems affect the description of echolocation calls. Poor descriptions could mask geographic variation within species making the discovery of cryptic species more difficult. Accurate representations of call parameters are also important for describing echolocation calls, for example in order to understand how signal design relates to ecology.

A number of studies of bat activity and habitat use have relied on subjective analysis of echolocation calls, in conjunction with observations of flight behaviour (Ahlén & Baagøe 1999; O'Farrell *et al.* 1999). The use of subjective analysis to separate species should be avoided, as surveys involving subjective analyses are difficult to replicate (Betts 1998; Robbins & Baltzke 1999; Jones *et al.* 2000; Parsons *et al.* 2000; Parsons & Obrist 2003). Acoustic surveys must be quantitative and objective. A quantitative measure of acoustic similarity is crucial to any study comparing the vocalisations of different species, social groups or individuals (Deeke *et al.* 1999). Objectivity is particularly important not only to eliminate the problems already mentioned but also to control for differences in the identification abilities among

recording equipment (Barclay 1999; Jones *et al.* in press). It is clear that there is a need for a systematic study of the effect of recording equipment on call descriptions.

In the present study, TE and FD were compared to determine if major differences in call description are generated by these frequency reduction techniques, and the probable causes of any differences. The two main methods of transforming the signal into the frequency-time domain, so that frequency parameters can be measured, were also compared for each frequency reduction technique. These were Fast Fourier Transformation (FFT) and zero-crossing analysis (ZCA). I aimed to investigate the effect of frequency reduction technique and transformation method on call parameters, as well as the effect of frequency reduction technique, transformation technique and species on each parameter.

2.1.2. Time expansion systems

TE involves the digital time expansion of a call to a lower frequency. The TE technique is based on the inverse relationship that exists between time and frequency. If the duration of a call is increased, the frequencies within the signal decrease (Parsons & Obrist 2003). The high frequency output from a microphone or bat detector is digitised at high sampling rates. The call is then converted back to an analogue waveform using reduced sampling rates, which results in increased signal duration. With TE, time-amplitude representations of the original calls are produced giving accurate details about frequency and harmonics (Jones 1991; Ahlén & Baagøe 1999; Jones *et al.* 2000; Parsons & Obrist 2003). The original call is time-expanded, normally by a factor of ten.

The main method used to transform TE calls into the frequency time-domain is Fourier analysis. The Fourier transform multiplies the original waveform with an artificial waveform of a particular frequency. The results are then summed over a range

of frequencies. The analysis constructs the artificial waveform so that both frequency and phase match those of the original waveform (Parsons *et al.* 2000). This results in a visual display of frequency, time and amplitude known as a spectrogram or sonogram.

An important benefit of TE is that virtually no information is removed from the call during the process (Jones *et al.* 2000). The main drawback of the TE technique is the fact that no new call can be acquired whilst a call is being time expanded (typically two seconds recording time followed by 20 seconds TE), and that the system is expensive due to the technology involved. The limited sampling time means that continuous recording using this technique is impossible, so that calls may be missed. Using a time expansion factor of 10 times, the system samples only nine percent of the available time (Vaughan *et al.* 1997a, Jones *et al.* 2000).

2.1.3. Frequency division systems

Frequency division (FD) reduces the frequency of the incoming signal by a predetermined ratio, normally 1:10, thereby lowering its frequency. A zero crossing system counts the number of times the waveform crosses a zero voltage level and converts the signal into a sine or square wave (Parsons *et al.* 2000). If a ratio of 1:10 is used, the system counts the number of zero crossings and lets information through on the tenth cycle (Fig. 2.1). This means that effectively 90% of the information from the original signal is lost. As the zero crossing system tracks the harmonic with the greatest amplitude, only information about the strongest harmonic in any signal is displayed. Fenton (2000) found that because of this fact, the echolocation calls of some species when in the presence of others were not detectable. FD signals are usually transformed into the frequency-time domain using zero-crossing analysis (ZCA). After digitisation, the time between successive points when the waveform crosses the average amplitude

level of the signal is measured. Because the time between successive crossings is related inversely to half the frequency of the signal at that point, a frequency-time representation of the signal can be created (Parsons *et al.* 2000).

The benefits of FD are that it is inexpensive compared with TE. Like TE it is broadband and therefore is ideal in survey work where a large number of frequencies need to be monitored. However, unlike TE, FD can be used to sample for long periods in real-time, therefore giving accurate indices of bat activity. Sites can also be monitored remotely (Barclay 1999). This system, however, does not give accurate representations of the original signal, and produces unrepresentative output for those species that vary the harmonic content of their calls, making it unsuitable for identification of such species (Fenton *et al.* 2001).

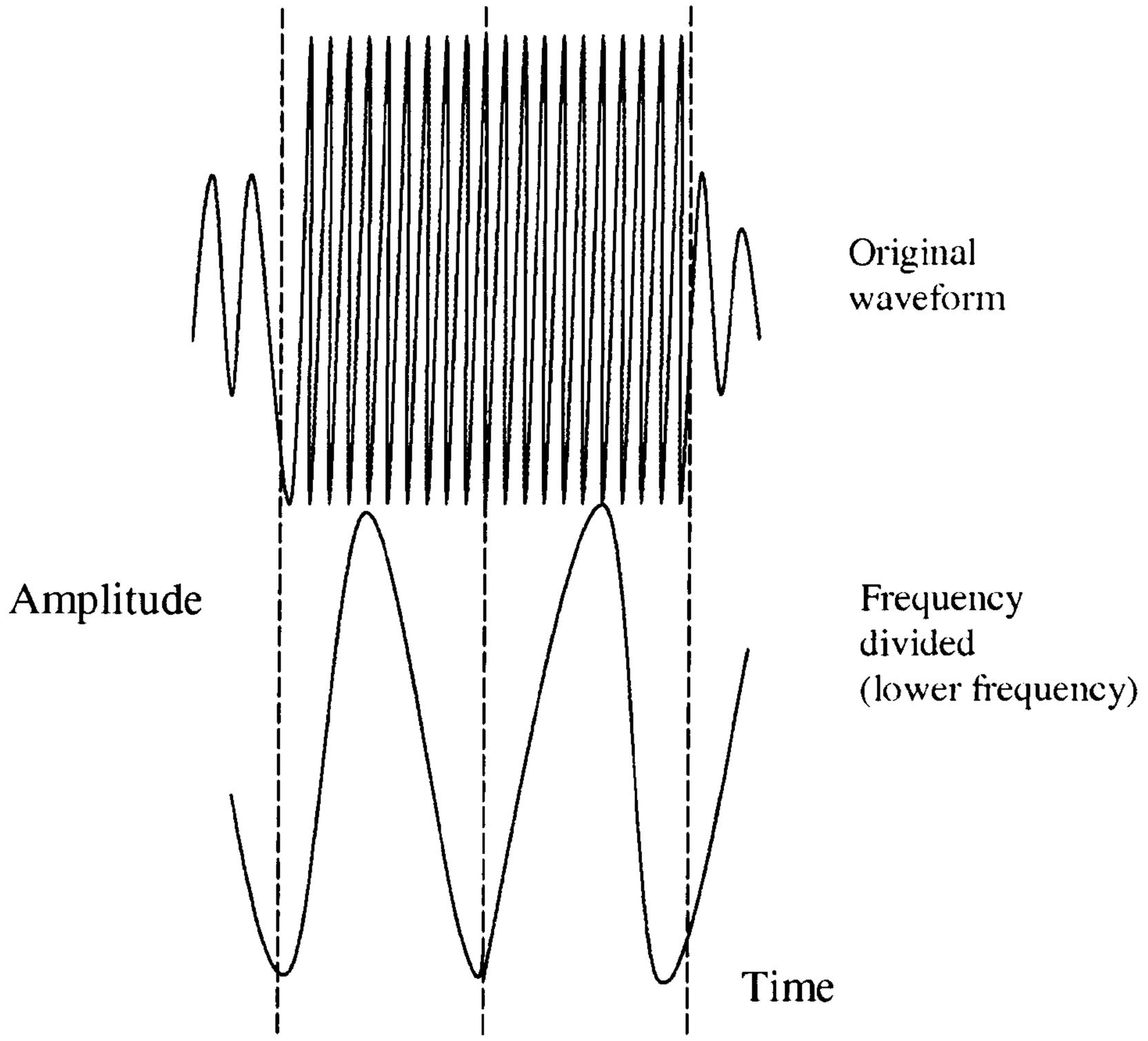
2.2 Methods

2.2.1. Recording of TE and FD calls

Search phase calls (Griffin 1958) were recorded from 12 British species of bat in 2001, at previously identified roost sites in southern England.

The flight lines from each roost site were known, and the equipment was set up bisecting one of these lines so the incoming signal would be on a direct axis with the bat detector microphone, housed on a tripod approximately one meter above the ground, angled at 45°.

Fig. 2.1 Simplified schematic of the frequency division process, using a ratio of 10:1.
The zero-crossing system counts the number of zero crossings and lets information through on the tenth cycle.



Free flying bats were recorded in this way whenever possible. In addition, bats released from the hand were recorded. These bats had been captured in mist nets or harp traps at roost sites and identified in the hand. Each bat was released from the hand four meters away from, and on axis with the microphone, and its calls were recorded.

The equipment used to record the calls consisted of a bat detector (S25, Ultra Sound Advice, London, UK) set to frequency division (ratio 10:1), (frequency response of microphone 20-120 kHz ±3dB). FD calls were recorded onto a digital audio tape (DAT, TCD-D8, Sony, Tokyo) recorder. Two seconds of the same call sequence was sampled from the high frequency (untransformed) output channel of the detector at 448 kHz with 8-bit precision and time-expanded by a factor of 10 using a Portable Ultrasound Processor (PUSP, Ultrasound Advice) before being recorded onto the DAT.

This technique resulted in stereo recordings of both FD and TE calls from the same call sequence of an individual bat, using the same recording equipment. Having the same microphone recording both TE and FD was an advance on a previous comparison (Fenton *et al.* 2001) in which systems with different microphones complicated the interpretation of any differences caused by the two analysis methods.

Calls were transferred to sound analysis software (BatSound v. 2, Pettersson Elektronik AB, Uppsala, Sweden) and advanced mathematical software (Matlab v. 6, The Mathworks Inc., Natick, MA, U.S.A.) for further analysis.

2.2.2. Measurement of time and frequency parameters

In BatSound, TE calls were displayed on one channel and FD calls on the other channel. A single call was selected with a good signal to noise ratio from the TE and FD recordings of each sequence, labelled and saved in a folder for subsequent analysis. Care was taken to identify the same call from both channels so that a TE and a FD recording of the same call from an individual bat could be compared (Figs. 2.2 and 2.3). This procedure was repeated for all the sequences recorded from various individuals. From here onwards, TE and FD calls will be referred to as TE frequency reduction technique or FD frequency reduction technique respectively.

FFT analysis

Two programs were written by Stuart Parsons using Matlab software for the objective, automated measurement and extraction of call parameters from both TE and FD frequency reduction techniques based on a Fast Fourier Transformation analysis (FFT). A mid-pass (10-150 kHz) tenth-order Butterworth filter was applied to the signals and the envelopes of each signal were calculated using a Hilbert transform of the waveform.

Envelopes were scaled between zero and one. Using the point at which the envelope crossed an arbitrary threshold value (0.0005), the call was removed from the rest of the signal (Parsons *et al.* 2000). A number of call parameters were then measured (Fig. 2.3). Call duration (Dur, ms) was defined as the duration of the extracted waveform (Parsons *et al.* 2000). The frequency with the most energy (FmaxE, kHz) was measured from a power spectrum derived from the extracted waveform. All the frequency measurements - start frequency (F-start, kHz), end frequency (F-end, kHz), and central frequency (C-freq, kHz; frequency at half the duration) - were taken from the two call types using comparable analyses. Using Fast Fourier Transformation (zero-padded 1024-point FFT), the frequency time course was plotted for both frequency reduction techniques by dividing the call into a series of 56-point segments. Power spectra were calculated for each segment and the frequency with most energy was calculated within each power spectrum. The extraction of the frequency measurements resulted from this sliding power spectrum (Jones & Parsons 2001).

ZCA analysis

The same calls were also analysed using the ZCA option in BatSound. Measurements from both frequency reduction techniques were taken manually. The detection threshold was set at zero percent, the nearest value to the 0.005 threshold used in the FFT scripts. The frequency calculations were made only for the portion of the signal exceeding this threshold. The number of samples in ZCA, which determines the averaging time of the analysis, was set to 50.

Fig. 2.2 Left (FD) and right (Fig. 2.3; TE) channels of the simultaneous recordings *of Myotis daubentonii.* The pattern of repetition is highlighted by the dashed line on both channels. A indicates the call selected for parameter extraction.

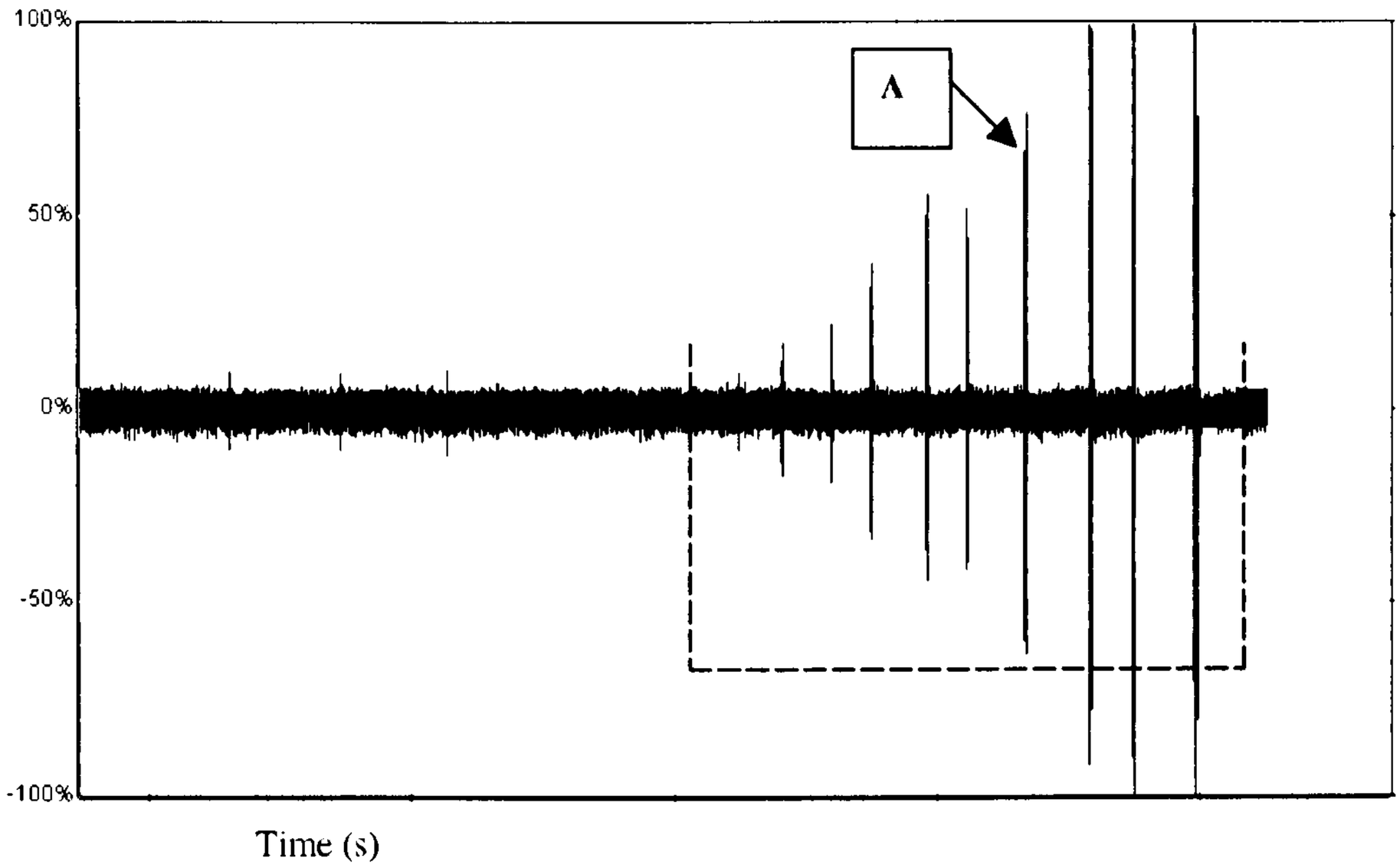


Fig. 2.3

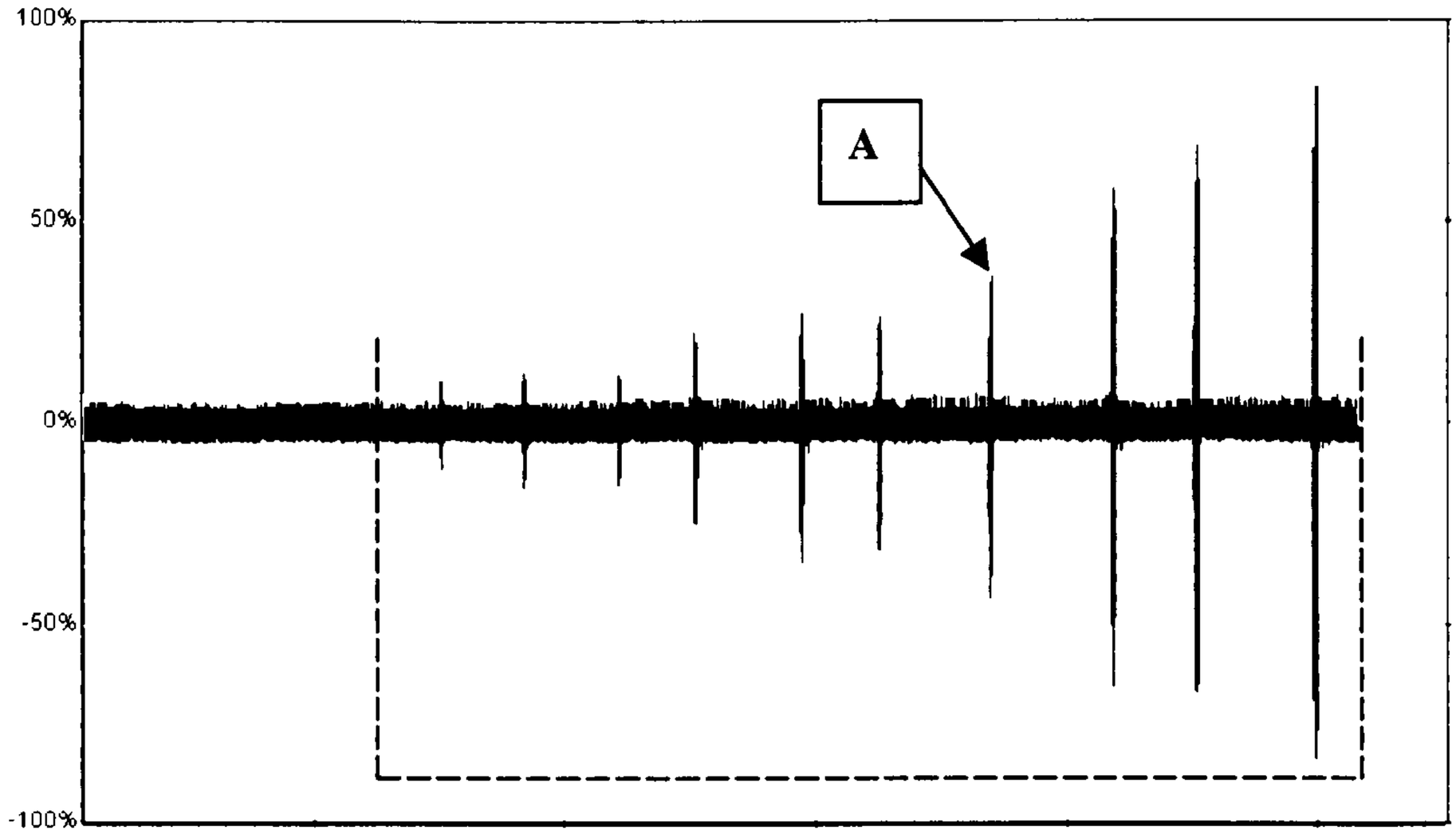
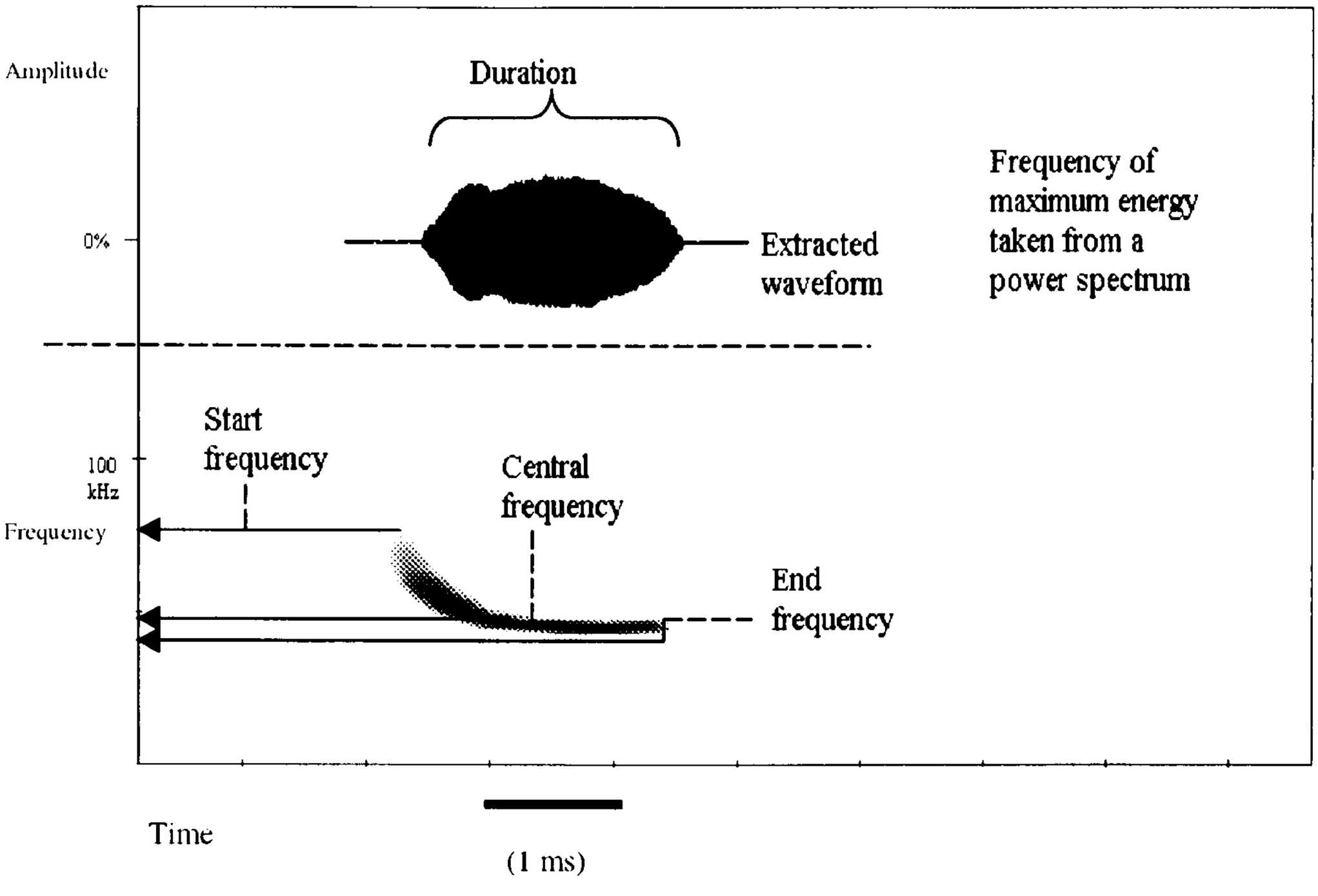


Fig. 2.4 Spectrogram of a *P. pipistrellus* call indicating the call parameters measured from each call.



The higher the number of samples in ZCA, the smoother the diagram produced. Duration was again measured from the extracted waveform. Using the measurement cursor, frequency measurements were taken from the visible curve on the output. Central frequency was measured at the point of half the duration. Frequency of most energy was measured using a power spectrum created using a 1024-point FFT in conjunction with a Hanning window.

2.2.3. Statistical methods

The data were first explored for general trends. To determine the overall effect of the two factors of frequency reduction and transformation techniques on call parameters, regardless of species, a repeated measures analysis of variance (ANOVA) was performed on the mean values of the parameters. Frequency reduction and

transformation techniques were the within-subject factors. Next, species differences were investigated. The overall effect of frequency reduction and transformation techniques on call parameters for different species was determined by a mixed design repeated measures ANOVA, with species as a between-subjects factor. Sphericity (Mauchly's test) and homogeneity of variance (Levene's test) were checked and correction values used where needed (Field 2000). Parameters for which there were significant effects of frequency reduction technique, transformation technique or species were submitted to a doubly multivariate repeated measures model, which considered all the parameters in one test.

The alpha value used throughout was 0.05. A significant result for frequency reduction*transformation indicates a significant interaction effect between frequency reduction and transformation technique on the call parameter, i.e. the effect of call type on the call parameter was different for the two techniques. The interactions can be interpreted from the estimated marginal mean plots, in which the direction of the interaction can be seen.

Most of the variables measured from the echolocation calls did not conform to multivariate normality (Box's M, F=13.174, P<0.001). However, ANOVA is a robust method that can cope with minor deviations from normality (Field 2002). For the mixed ANOVA, violation of sphericity was corrected for by using Greenhouse-Geisser correction values (Field 2002).

2.3 Results

2.3.1. Effect of frequency reduction technique when calls are transformed by FFT

The mean and the standard error of call parameters of both frequency reduction techniques analysed by the FFT extraction method, are shown in Tables 2.1a and b.

The results from the FFT technique of the TE call parameters are in general agreement with those in the current literature (Vaughan *et al.* 1997b; Parsons & Jones 2000). FD calls have on average higher pulse duration, higher start and end frequencies, lower frequency of maximum energy and lower central frequency than TE calls. The FD calls analysed using FFT are more broadband than the TE calls. When compared to the TE/FFT data there are some oddities, notably increased values for start frequency for *Plecotus auritus*, *Nyctalus noctula* and *Nyctalus leisleri*, low values for F-end for *Pipistrellus pygmaeus*, and very low values for all parameters except duration for *Rhinolophus ferrumquinum*.

Table 2.1 a Descriptive statistics for TE calls. Parameters extracted using the FFT method. Mean±SEM.

	n	**Dur (ms)**	**F-start (kHz)**	**F-end (kHz)**	**F-maxE (kHz)**	**C-freq (kHz)**
Rhinolophus ferrumequinum	16	42.34±3.35	82.43±1.18	70.46±2.75	81.71±0.34	79.33±2.58
R. hipposideros	18	37.09±3.11	109.00±3.15	89.61±6.20	110.11±0.34	107.28±3.08
Myotis bechsteinii	35	1.57±0.08	102.42±2.38	41.90±0.95	66.97±1.66	76.20±1.50
M. nattereri	23	1.61±0.12	110.25±2.32	36.99±1.11	66.51±2.31	82.29±2.26
M. mystacinus	25	2.25±0.13	95.70±1.96	39.71±1.09	53.43±1.10	65.51±1.73
M. brandtii	12	2.13±0.10	97.60±2.29	38.68±0.68	56.08±1.48	64.57±2.37
M. daubentonii	35	1.88±0.10	79.81±1.98	38.47±0.62	54.00±0.93	58.28±0.99
Pipistrellus pygmaeus	37	5.81±0.20	69.26±1.37	50.73±0.39	51.76±0.39	52.13±0.40
Nyctalus noctula	27	11.71±0.86	35.94±1.96	21.80±0.68	24.40±0.73	24.27±0.86
N. leisleri	33	7.12±0.31	53.56±1.95	27.57±0.32	30.41±0.58	31.20±0.61
Eptesicus serotinus	35	6.83±0.28	57.5±1.53	27.23±0.25	32.41±0.45	33.53±0.44
Plecotus auritus	11	2.45±0.33	51.61±2.55	33.33±2.17	40.79±2.85	41.00±2.47

Table 2.1 b Descriptive statistics for FD calls. Parameters extracted using the FFT method. Mean±SEM.

	n	Dur (ms)	F-start (kHz)	F-end (kHz)	F-maxE (kHz)	C-freq (kHz)
Rhinolophus ferrumequinum	16	47.51±2.155	53.37±6.15	25.44±0.83	25.44±1.63	32.31±2.05
R. hipposideros	18	47.01±1.75	112.14±3.47	76.96±5.02	108.00±0.79	104.93±3.37
Myotis bechsteinii	35	3.82±0.30	116.73±3.35	38.89±1.45	59.63±2.26	82.30±3.06
M. nattereri	23	3.03±0.27	92.87±8.01	33.87±2.19	50.63±3.64	71.03±6.48
M. mystacinus	25	3.13±0.17	97.86±3.26	37.21±1.58	50.14±1.51	63.94±2.83
M. brandtii	12	3.16±0.23	89.59±8.57	33.88±3.22	44.21±4.68	53.98±6.16
M. daubentonii	35	3.59±0.25	73.24±4.28	32.91±1.94	42.14±2.76	48.83±3.04
Pipistrellus pygmaeus	37	6.48±0.15	73.56±2.64	39.66±1.54	49.69±0.54	52.30±0.94
Nyctalus noctula	27	12.10±0.68	66.60±4.81	24.02±0.67	23.51±0.84	32.19±1.04
N. leisleri	33	7.44±0.27	70.99±2.47	25.72±0.62	30.53±0.60	34.48±1.03
Eptesicus serotinus	35	7.66±0.29	66.92±1.78	24.64±0.59	30.36±0.58	35.46±0.85
Plecotus auritus	11	4.46±0.56	75.16±8.13	31.97±2.82	43.22±4.55	46.99±5.07

2.3.2. Effect of frequency reduction technique when calls are transformed by ZCA

The mean and the standard error of call parameters measurements of both call types for the ZCA analysis method are shown in Tables 2.2 a and b.

The parameters derived from TE calls analysed using ZCA are generally in agreement with the TE/FFT results and therefore the published literature. The only strange result is the increased value for *Plecotus* start frequency. Most of the FD/ZCA results vary with either higher or lower values compared to the published data.

Table 2.2 a Descriptive statistics for TE calls. Parameters extracted using the ZCA method. Mean±SEM

	n	Dur (ms)	F-start (kHz)	F-end (kHz)	F-maxE (kHz)	C-freq (kHz)
Rhinolophus ferrumequinum	16	52.37±2.97	74.32±0.87	73.95±0.74	82.20±0.33	82.20±0.33
R. hipposideros	18	47.83±2.28	98.42±1.33	97.17±1.24	110.37±0.37	110.37±0.37
Myotis bechsteinii	35	2.02±0.09	111.00±2.21	35.75±0.38	65.78±2.12	70.88±1.77
M. nattereri	23	2.49±0.16	131.20±2.08	33.80±1.22	62.10±2.03	71.32±4.20
M. mystacinus	25	2.82±0.16	105.93±2.39	39.71±1.09	56.25±1.17	59.87±1.80
M. brandtii	12	2.67±0.14	115.44±2.83	35.40±1.11	53.85±1.00	56.98±1.32
M. daubentonii	35	2.58±0.16	102.52±1.36	39.53±0.95	52.25±1.10	54.23±0.76
Pipistrellus pygmaeus	37	6.54±0.14	72.98±1.21	51.66±0.35	51.98±0.40	52.97±0.37
Nyctalus noctula	27	15.21±1.10	38.04±2.01	23.38±0.59	24.77±0.62	25.10±0.69
N. leisleri	33	8.24±0.34	61.74±2.13	28.62±0.34	30.59±0.60	32.81±0.52
Eptesicus serotinus	35	8.24±0.34	65.89±1.27	27.81±0.21	31.75±0.47	32.46±0.39
Plecotus auritus	11	3.48±0.50	60.81±1.91	29.81±0.94	39.30±2.93	39.27±2.66

Table 2.2 b Descriptive statistics for FD calls. Parameters extracted using the ZCA method. Mean±SEM

	n	Duration (ms)	F-start (kHz)	F-end (kHz)	F-maxE (kHz)	F-centre (kHz)
Rhinolophus ferrumequinum	16	50.5±2.50	43.90±1.58	42.41±2.30	29.30±1.71	32.59±1.93
R. hipposideros	18	50.3±1.76	97.84±1.66	95.58±2.30	113.79±0.33	113.79±0.33
Myotis bechsteinii	35	2.70±0.16	132.10±3.09	60.60±1.54	68.62±4.50	105.52±2.72
M. nattereri	23	3.8±0.43	125.20±5.51	52.59±4.39	61.37±6.50	76.80±7.15
M. mystacinus	25	3.80±0.21	110.20±2.58	47.96±2.09	46.86±1.54	64.27±3.62
M. brandtii	12	4.1±0.39	121.3±6.05	40.99±4.05	42.77±3.55	72.97±9.22
M. daubentonii	35	4.2±0.39	125.78±3.99	40.38±2.97	43.10±3.15	73.98±6.22
Pipistrellus pygmaeus	37	7.43±0.22	73.66±1.27	52.31±0.46	51.96±0.41	54.62±0.41
Nyctalus noctula	27	14.89±1.29	41.63±1.93	27.43±0.90	25.17±0.70	27.44±0.75
N. leisleri	33	9.64±1.30	101.33±4.50	31.22±0.47	40.00±8.26	34.64±0.56
Eptesicus serotinus	35	9.66±0.38	86.24±3.60	31.74±0.72	30.26±0.34	34.39±0.55
Plecotus auritus	11	5.1±0.81	111.6±8.93	37.34±6.03	44.25±3.93	68.35±9.35

2.3.3. Overall effects of frequency reduction and transformation method on call parameters

The mean of the mean values of each call parameter were calculated for each species, to get an overview of general trends (Figs 2.5 to 2.9). Results from the repeated measures ANOVA with frequency reduction technique and transformation method as within subject factors showed significantly higher overall values produced with method 2 (ZCA) compared to the FFT method for signal duration ($F_{1,11}$=8.692, P<0.05), start frequency ($F_{1,11}$=6, P<0.05) and end frequency ($F_{1,11}$=29.09, P<0.001). For duration FD calls also produced significantly higher values ($F_{1,11}$=13.02, P<0.05) compared with TE calls. Frequency reduction technique had no significant main effect on start frequency, end frequency, frequency of maximum energy and central frequency. There were significant interaction effects between frequency reduction and transformation method for end frequency ($F_{1,11}$=29, P<0.001) and frequency of maximum energy ($F_{1,11}$=7.7, P<0.05), meaning that the effect of transformation method differed depending on which frequency reduction technique was used. The estimated marginal mean plots indicate the direction of these interactions (Figs 2.10-2.12). With end frequency, FD analysed using FFT produced on average lower values compared with TE calls analysed the same way (Fig. 2.10), with frequency of maximum energy FFT produced higher values for TE calls compared to FD calls (Fig. 2.11) and with central frequency ZCA produced lower values with TE calls but higher values with FD calls (Fig. 2.12).

Fig. 2.5 General trends for duration with frequency reduction and transformation method.

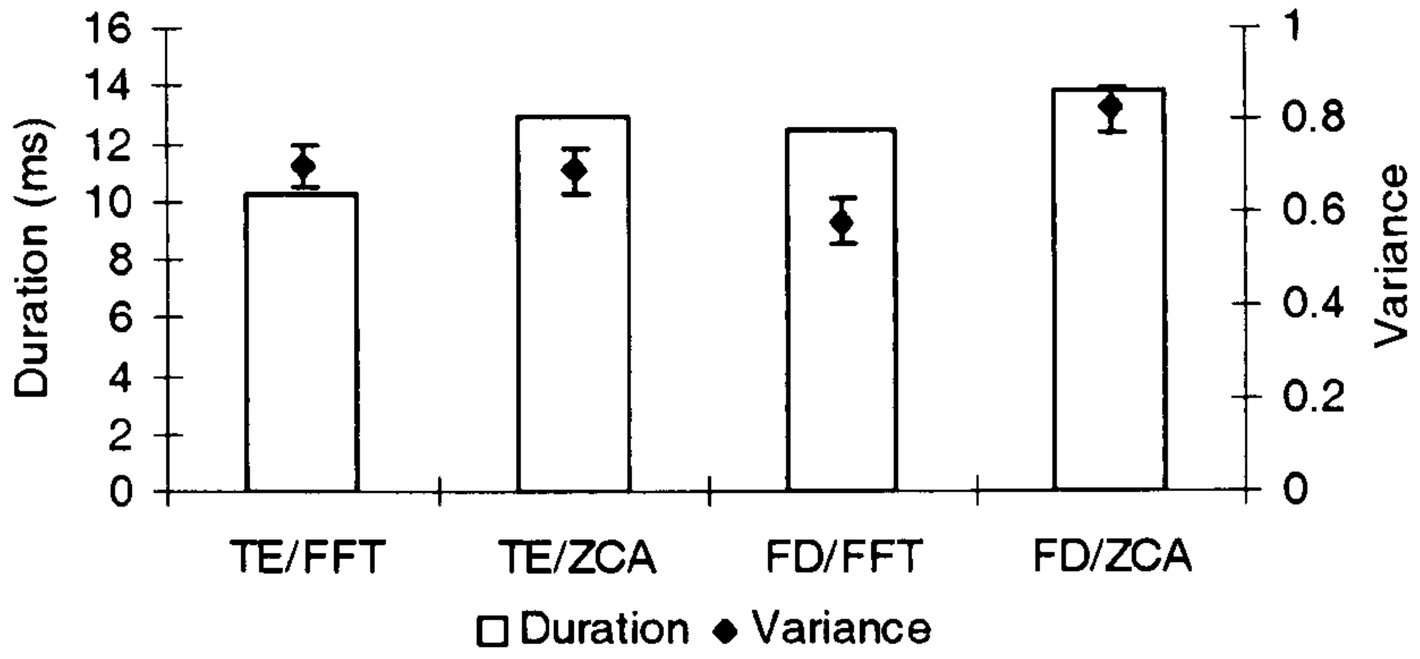


Fig. 2.6 General trends for start frequency with frequency reduction and transformation method.

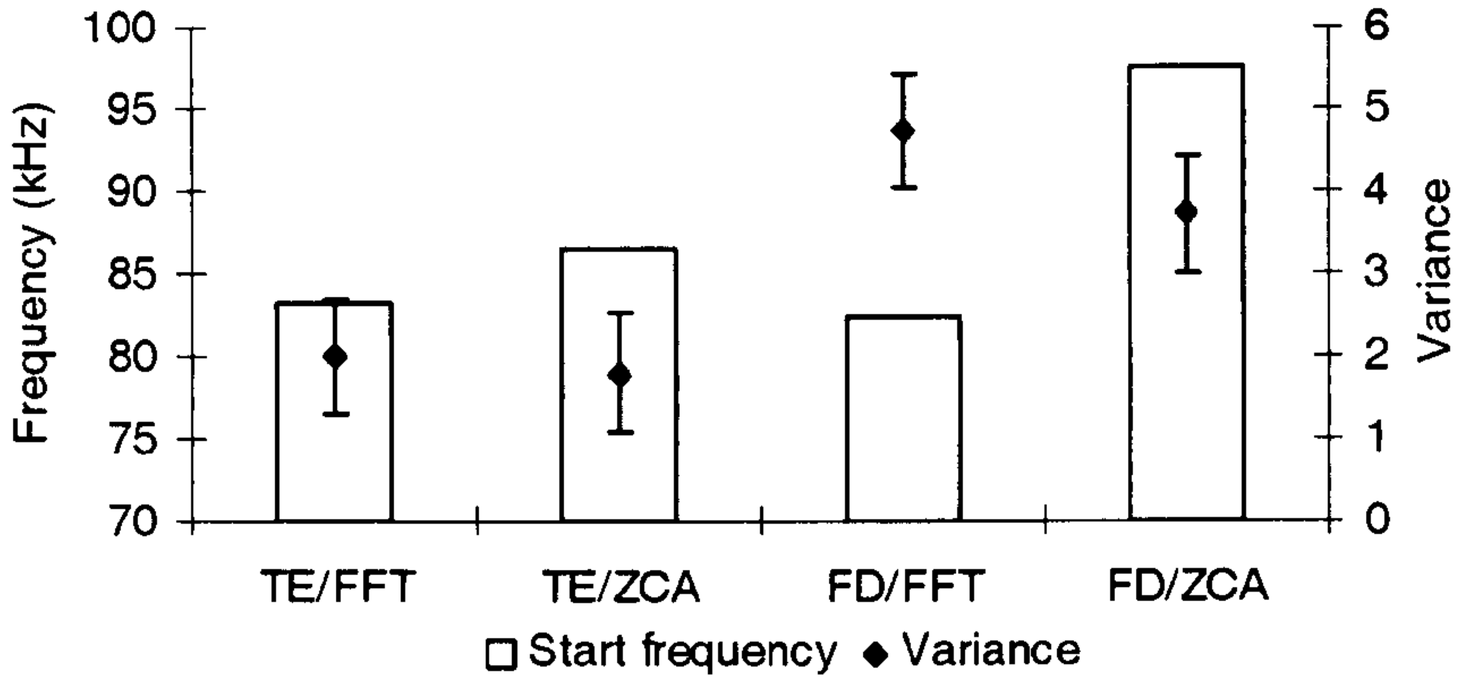


Fig. 2.7 General trends for end frequency with frequency reduction and transformation method.

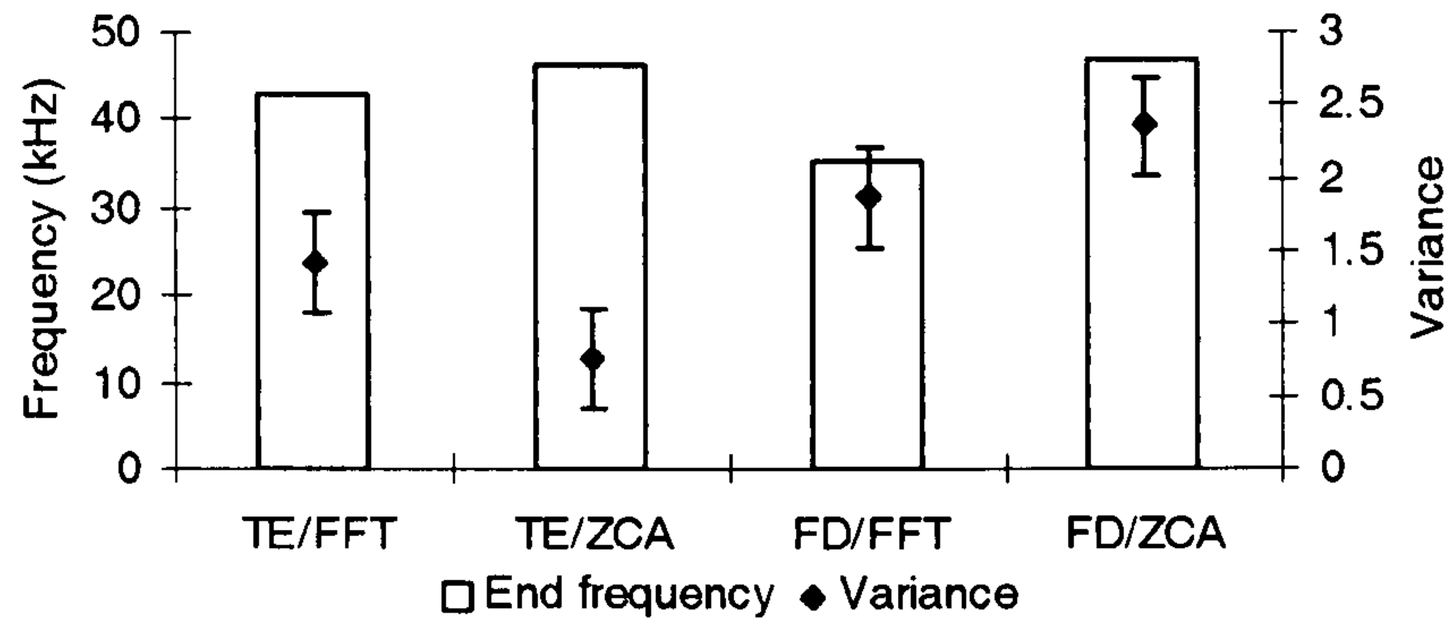


Fig. 2.8 General trends for frequency of maximum energy with frequency reduction and transformation method.

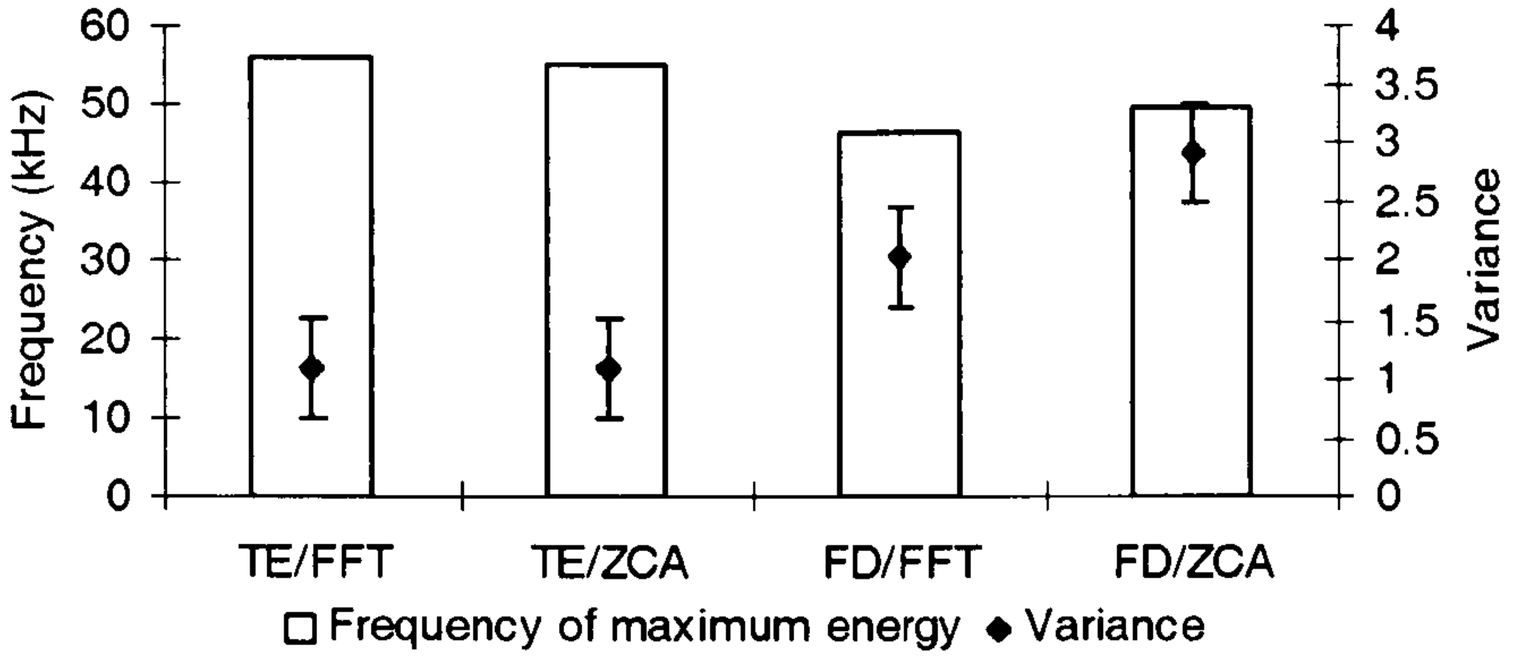


Fig. 2.9 General trends for central frequency with frequency reduction and transformation method.

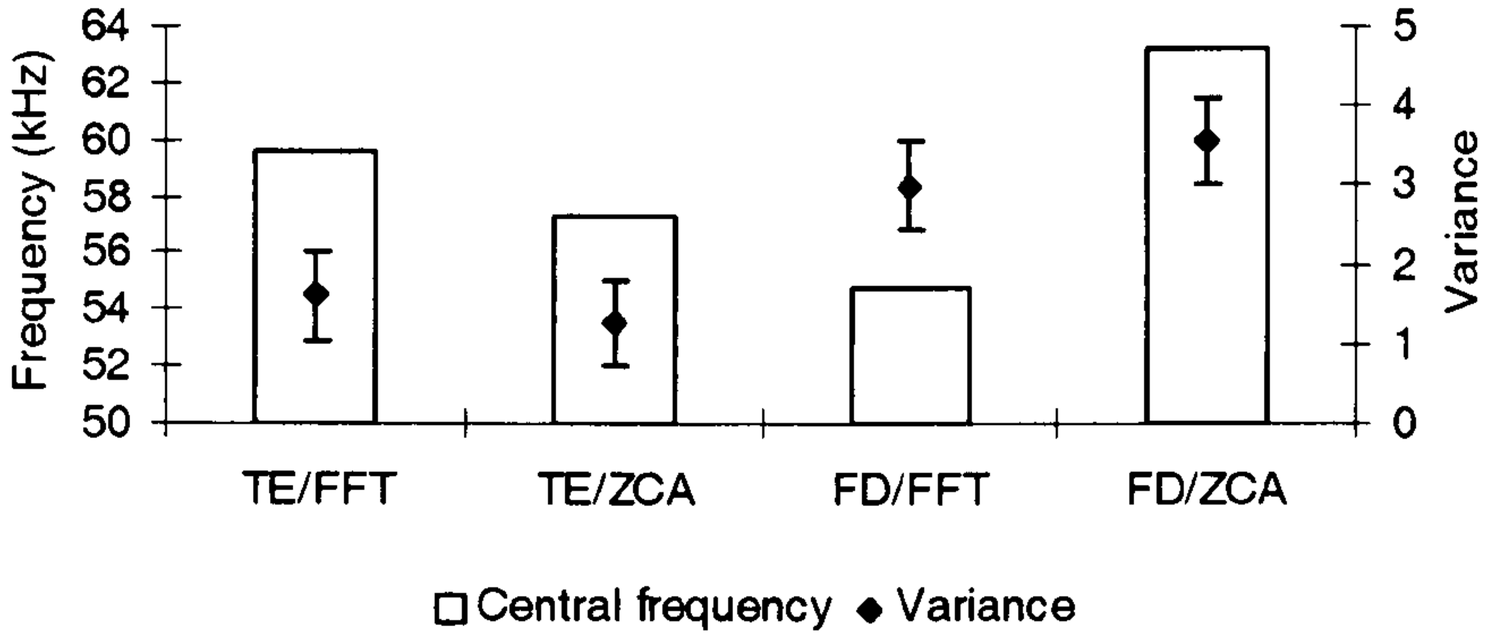


Fig. 2.10 Estimated marginal mean plots for the significant interaction effects of end frequency.

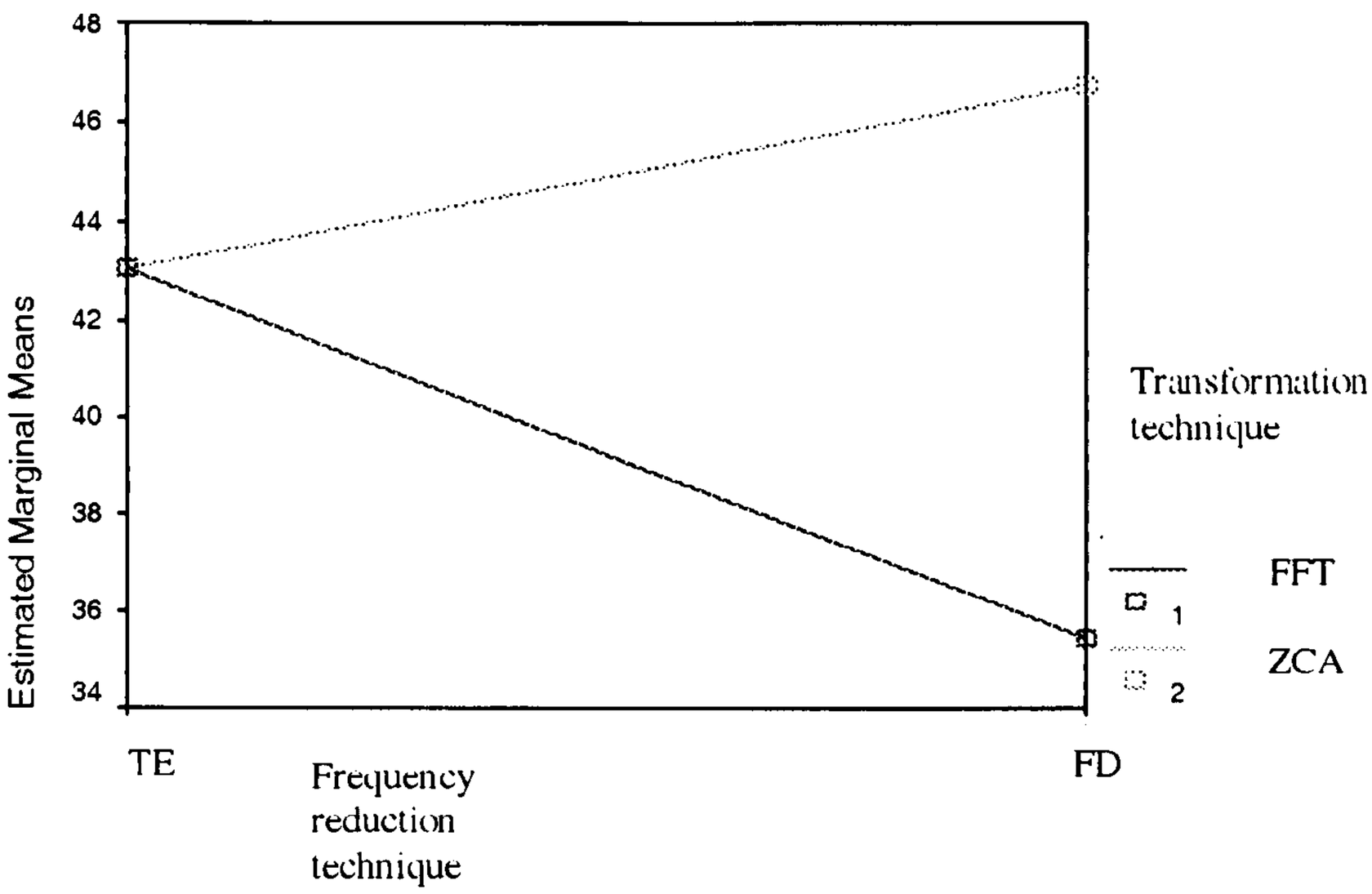


Fig. 2.11 Estimated marginal mean plots for the significant interaction effects of frequency of maximum energy.

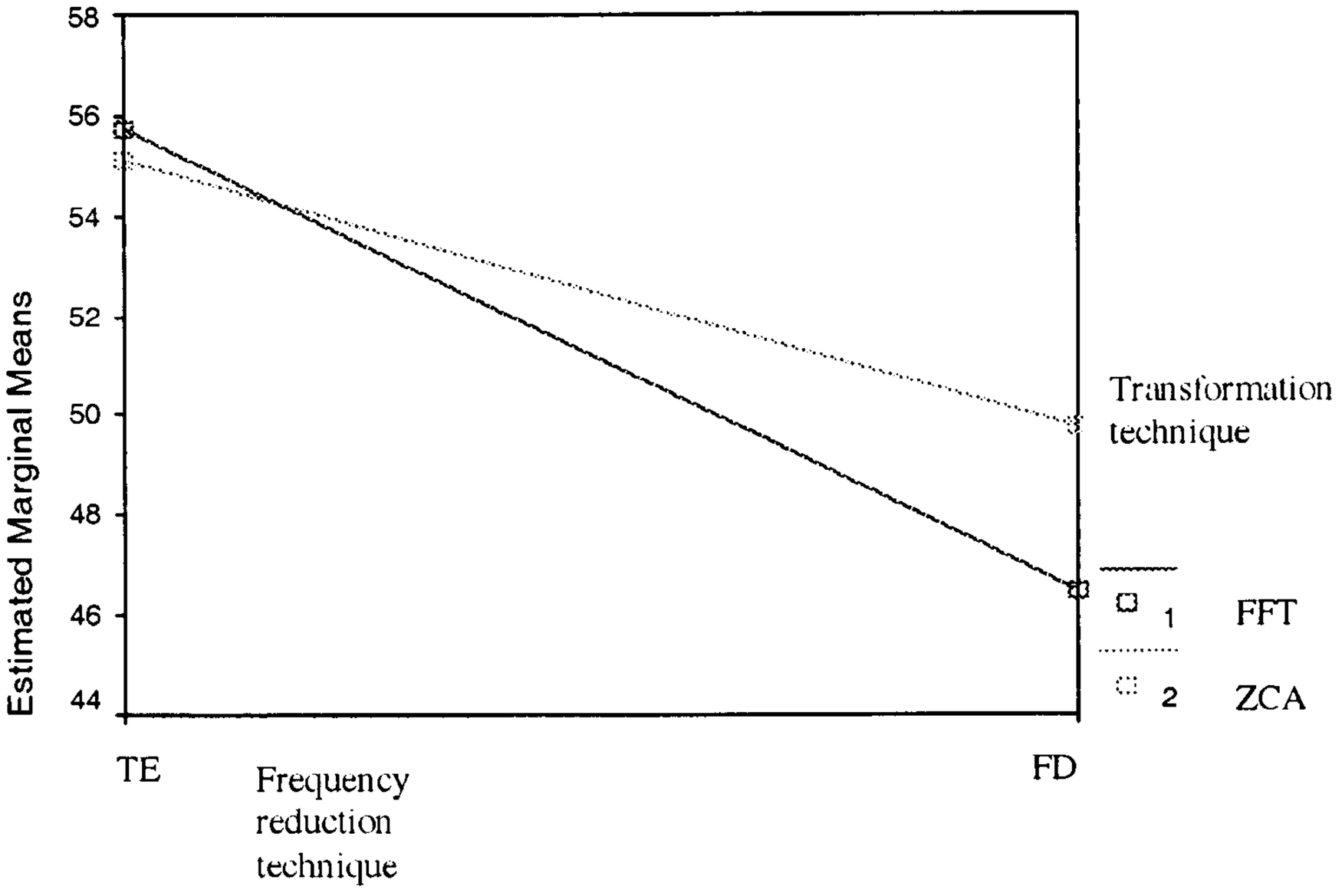
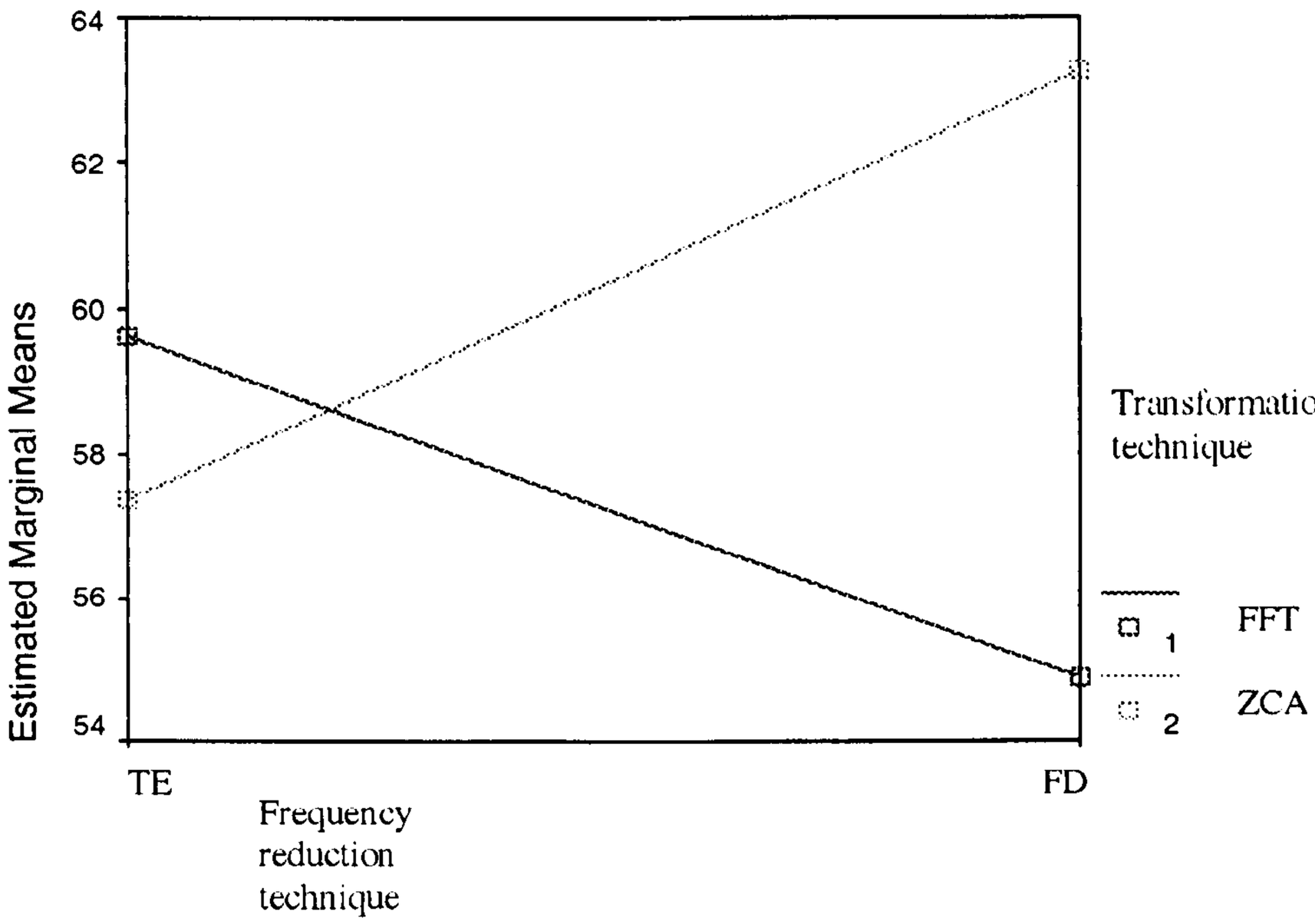


Fig. 2.12 Estimated marginal mean plots for the significant interaction effects of central frequency.



2.3.4. Effects of frequency reduction technique, transformation method and species on call parameters

Duration

Species (ignoring all other variables) had a significant main effect on duration indicating that the call parameter measures differed for different species, as expected (Table 2.3). Highly significant main effects ($P<0.001$) of frequency reduction and transformation techniques were seen for duration, indicating that the values of this parameter were significantly different depending on frequency reduction or transformation method used, regardless of each other. No significant interaction was evident between frequency reduction technique and species. This means that the effect of frequency reduction technique was the same for all species (Table 2.3). For all species, FD calls produced higher values compared to TE calls (Fig. 2.13). The significant interaction effect between transformation method and species ($P<0.001$) indicates that different species responded differently to the transformation method used (Fig. 2.14). The non-significant 3-way interaction between frequency reduction technique, transformation method and species means that the combined effects of all these variables (i.e. direction) was the same for all species. FFT transformation method produced lower values with both TE and FD calls compared to the ZCA transformation method.

Table 2.3 Repeated measures ANOVA on duration for within subject effects: frequency reduction and transformation technique, and between subject effect: species. Source =source of variation, df = degrees of freedom, * = interaction term.

Source	**df**	**F**	***P***
Species	9	111.51	<0.001
Frequency reduction	1	45.4	<0.001
Frequency reduction*species	9	1.05	0.398
Transformation	1	88.8	<0.001
Transformation*species	9	8.5	<0.001
Frequency reduction*transformation	1	0.049	0.825
Frequency reduction*transformation*species	9	1.6	0.116

Fig. 2.13 Interaction between species and frequency reduction technique for duration.

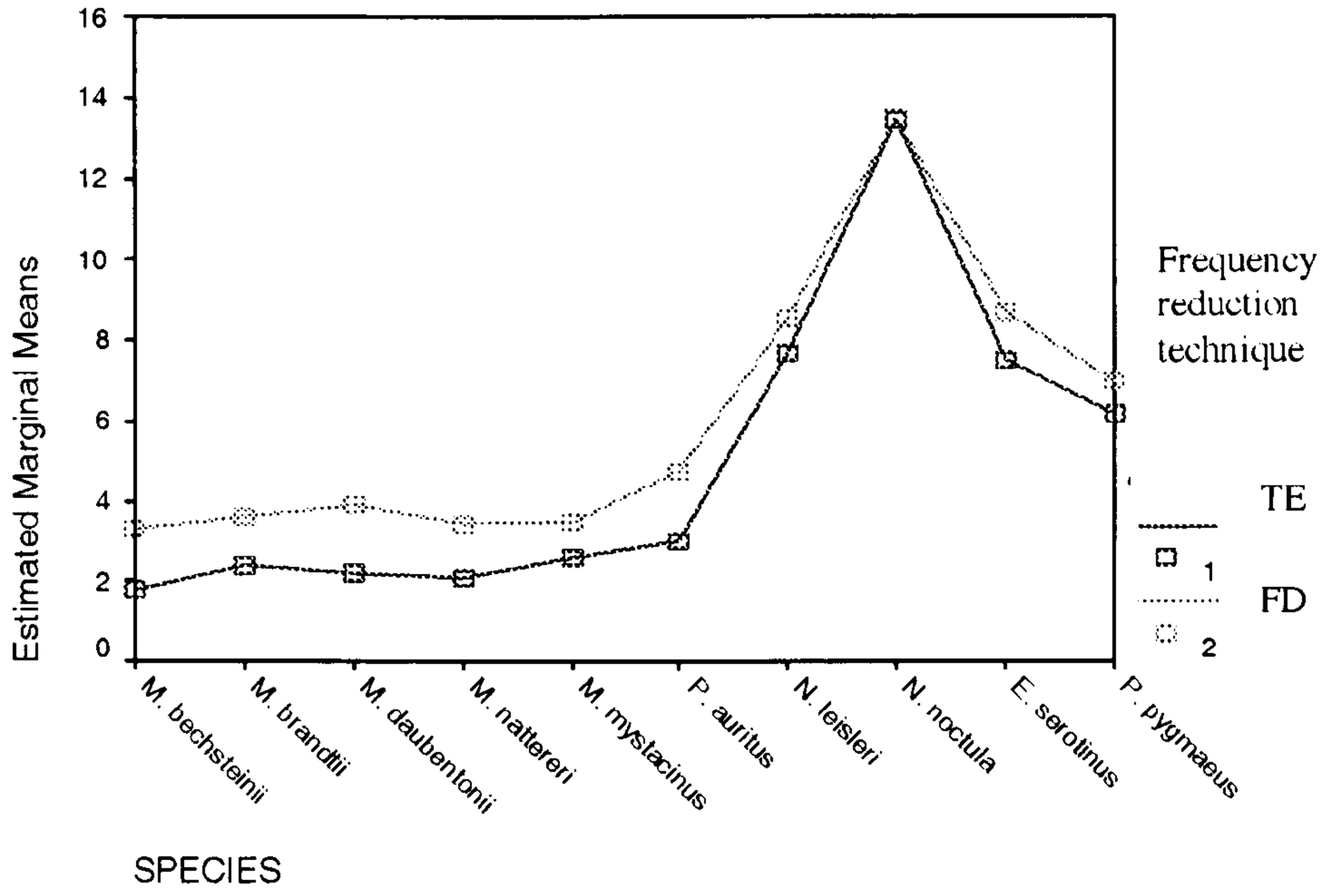
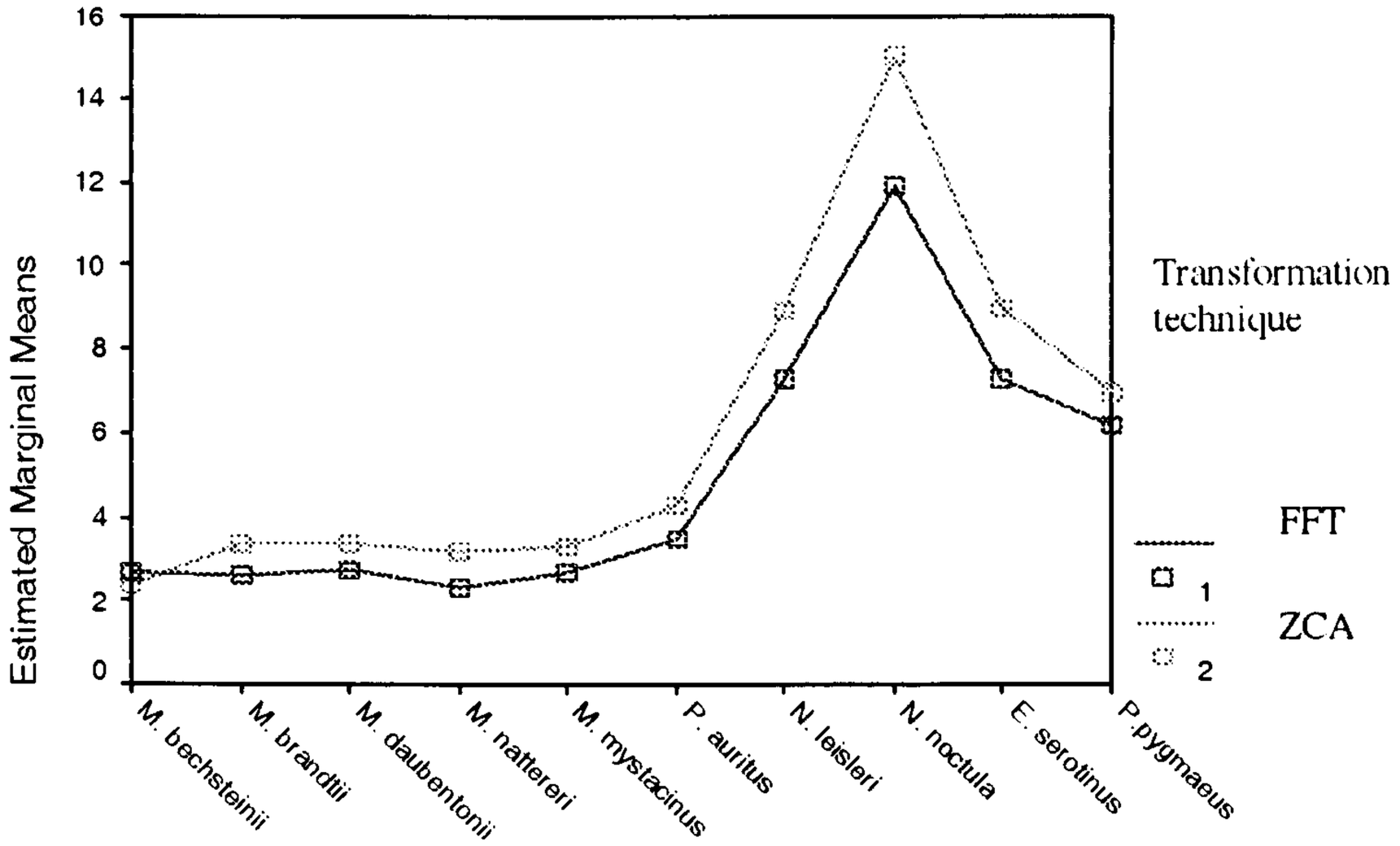


Fig. 2.14 Interaction between species and transformation method for duration.



Start frequency

Species (ignoring all other variables) had a significant main effect on start frequency indicating that the call parameter measures differed for different species (Table 2.4). Significant main effects ($P<0.001$) of frequency reduction and transformation technique were evident for this parameter indicating that the values of F-start were significantly different depending on frequency reduction or transformation method used (Table 2.4). The significant interaction effect for frequency reduction technique and species ($P<0.001$) indicates that the effect of frequency technique on F-start was different for different species. Figure 2.15 shows that FD calls produced lower values for *M. nattereri* but higher values for *M. bechsteinii*. Both frequency reduction techniques produced similar values for *M. brandtii*. The significant interaction between transformation method and species meant that the effect of transformation method used was different for different species ($P<0.001$, Fig. 2.16). FFT transformation method produced higher values for *N. noctula*, but lower values for all other species. The effect of transformation method was significantly different depending on the frequency reduction technique used ($P<0.001$). The significant 3-way interaction between frequency reduction technique, transformation method and species indicates that the combined effects of frequency reduction technique and transformation method were different for different species. FFT method of transformation on both frequency reduction techniques produced lower values compared to the ZCA method. *N. noctula*, however, had higher values when FFT was used to analyse FD calls compared to when FD calls were analysed by ZCA.

Table 2.4 Repeated measures ANOVA on start frequency for within subject effects: frequency reduction and transformation technique, and between subject effect: species. Source =source of variation, df = degrees of freedom, * = interaction term.

Source	df	F	*P*
Species	9	137.48	<0.001
Frequency reduction	1	88.6	<0.001
Frequency reduction*species	9	12.3	<0.001
Transformation	1	348.5	<0.001
Transformation*species	9	33.2	<0.001
Frequency reduction*transformation	1	34.4	<0.001
Frequency reduction*transformation*species	9	12.9	<0.001

Fig. 2.15 Interaction between species and frequency reduction technique for start frequency.

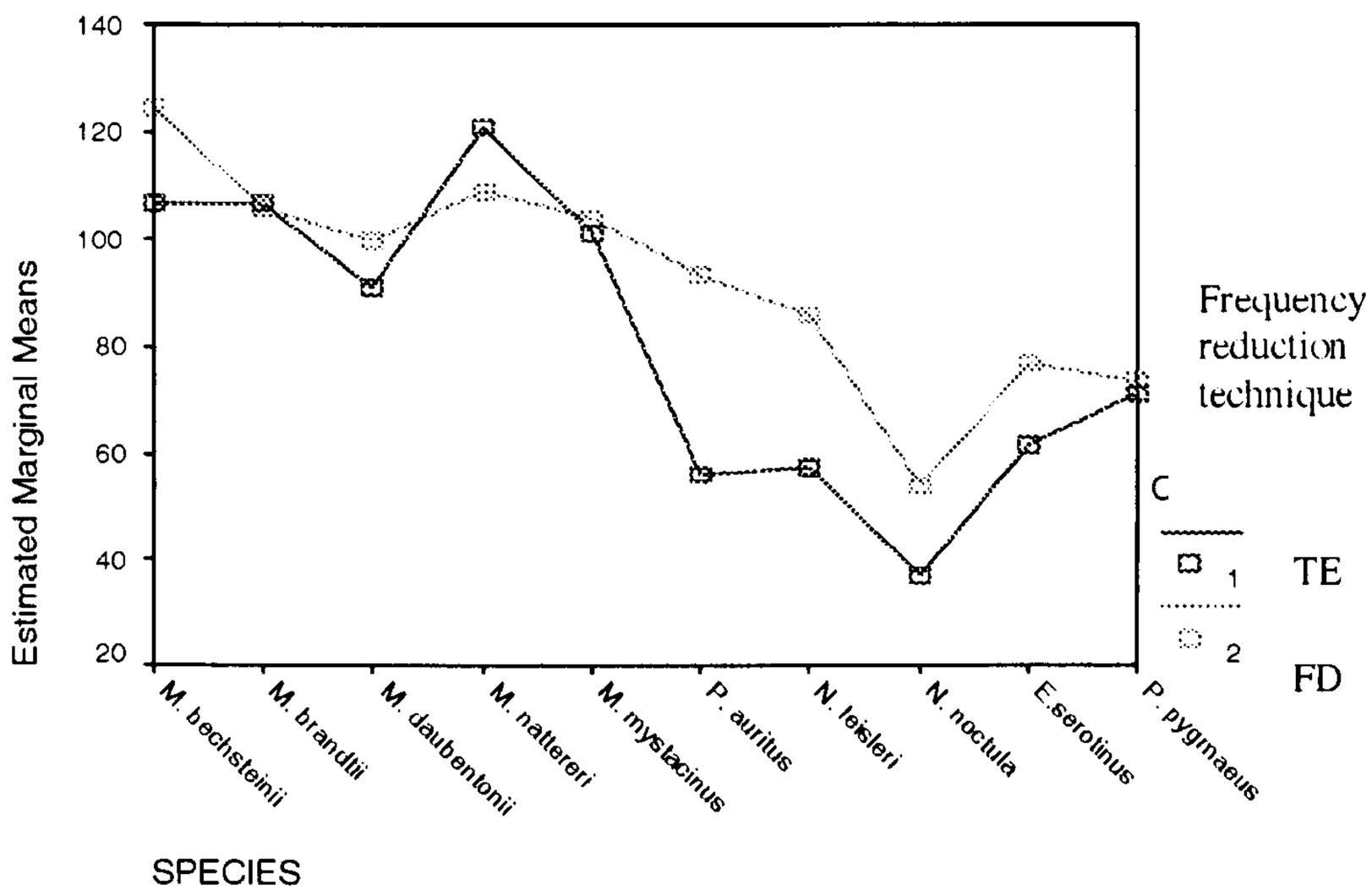
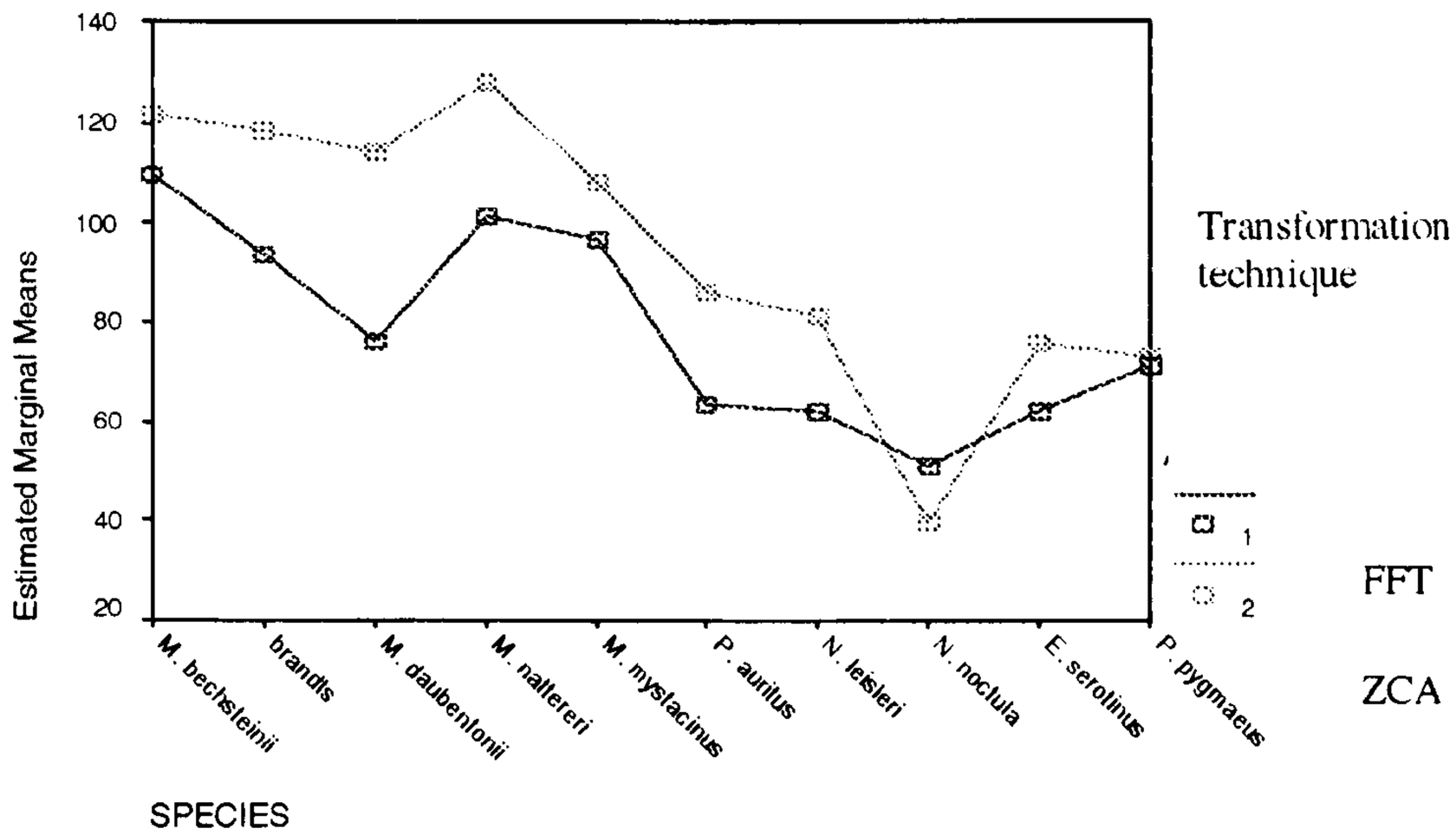


Fig. 2.16 Interaction between species and transformation method for start frequency.



End frequency

Species (ignoring all other variables) had a significant main effect on end frequency indicating that the call parameter measures differed for different species (Table 2.5). There were significant main effects ($P<0.001$) of frequency reduction and transformation method on this parameter, indicating that, regardless of either variable, the effect of frequency reduction/transformation method produced significantly different values for end frequency (Table 2.5). As with start frequency, all interactions were significant ($P<0.001$). The effects of frequency reduction technique (Fig. 2.17) and transformation method (Fig. 2.18) were significantly different for different species. TE frequency reduction technique produced higher values for *M. daubentonii* and *P. pygmaeus* but lower values for all other species. The effect of transformation method used differed significantly depending on which frequency reduction technique was used, and the combined effects of frequency reduction and transformation method was different for different species. FFT transformation method produced lower values for both TE and FD calls for *M. daubentonii*, *M. mystacinus*, *N. leisleri*, *N. noctula*, *E. serotinus* and *P. pygmaeus*. ZCA transformation method produced lower values through the analysis of TE calls than through the analysis of FD calls for *M. bechsteinii*, *M. brandtii*, *M. nattereri* and *P. auritus*.

Table 2.5 Repeated measures ANOVA on end frequency for within subject effects: frequency reduction and transformation technique, and between subject effect: species. Source =source of variation, df = degrees of freedom, * = interaction term.

Source	**df**	**F**	***P***
Species	9	73.32	<0.001
Frequency reduction	1	16.6	<0.001
Frequency reduction*species	9	11.1	<0.001
Transformation	1	179.7	<0.001
Transformation*species	9	5.3	<0.001
Frequency reduction*analysis	1	247.1	<0.001
Frequency reduction*transformation*species	9	17.5	<0.001

Fig. 2.17 Interaction between species and frequency reduction technique for end frequency.

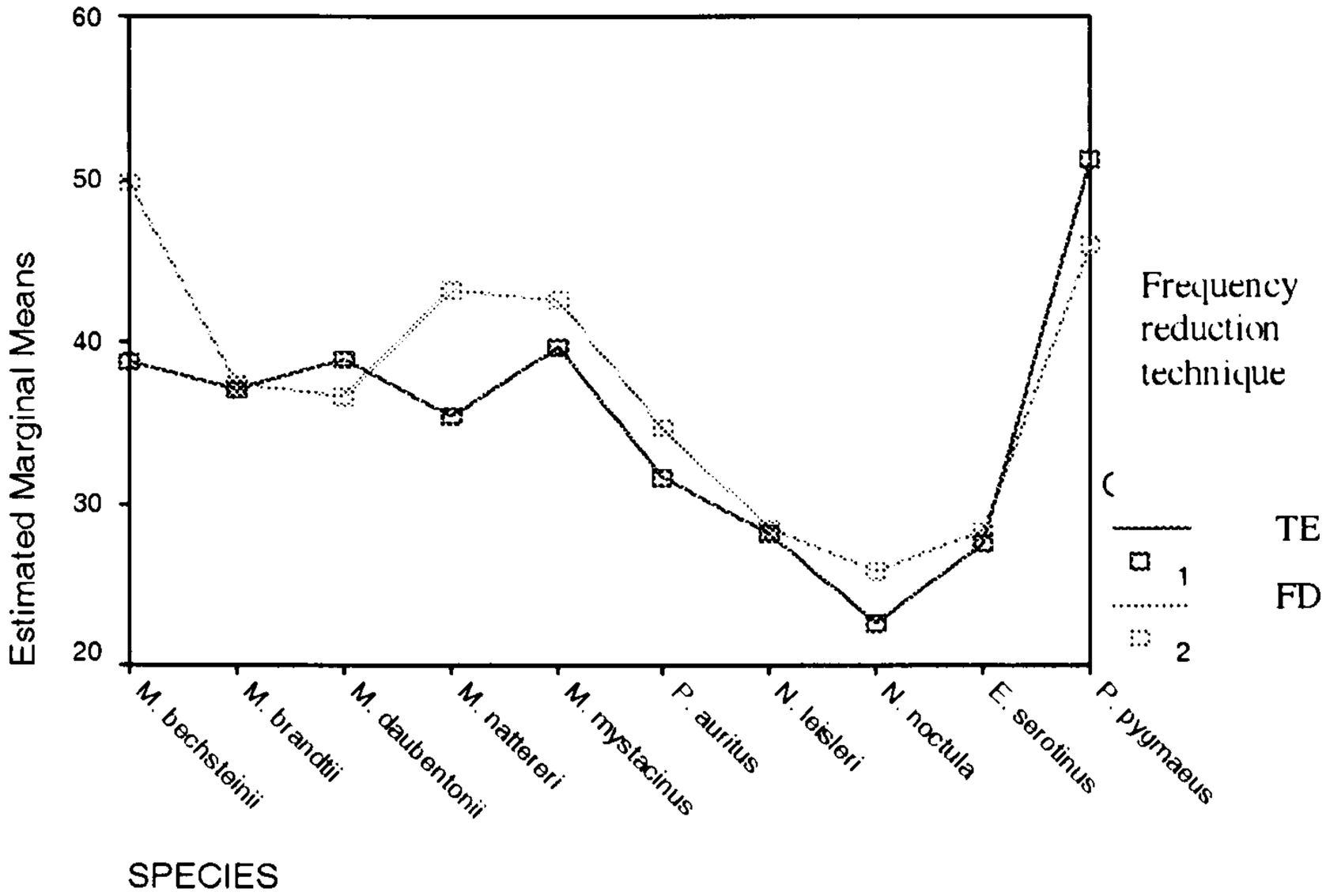
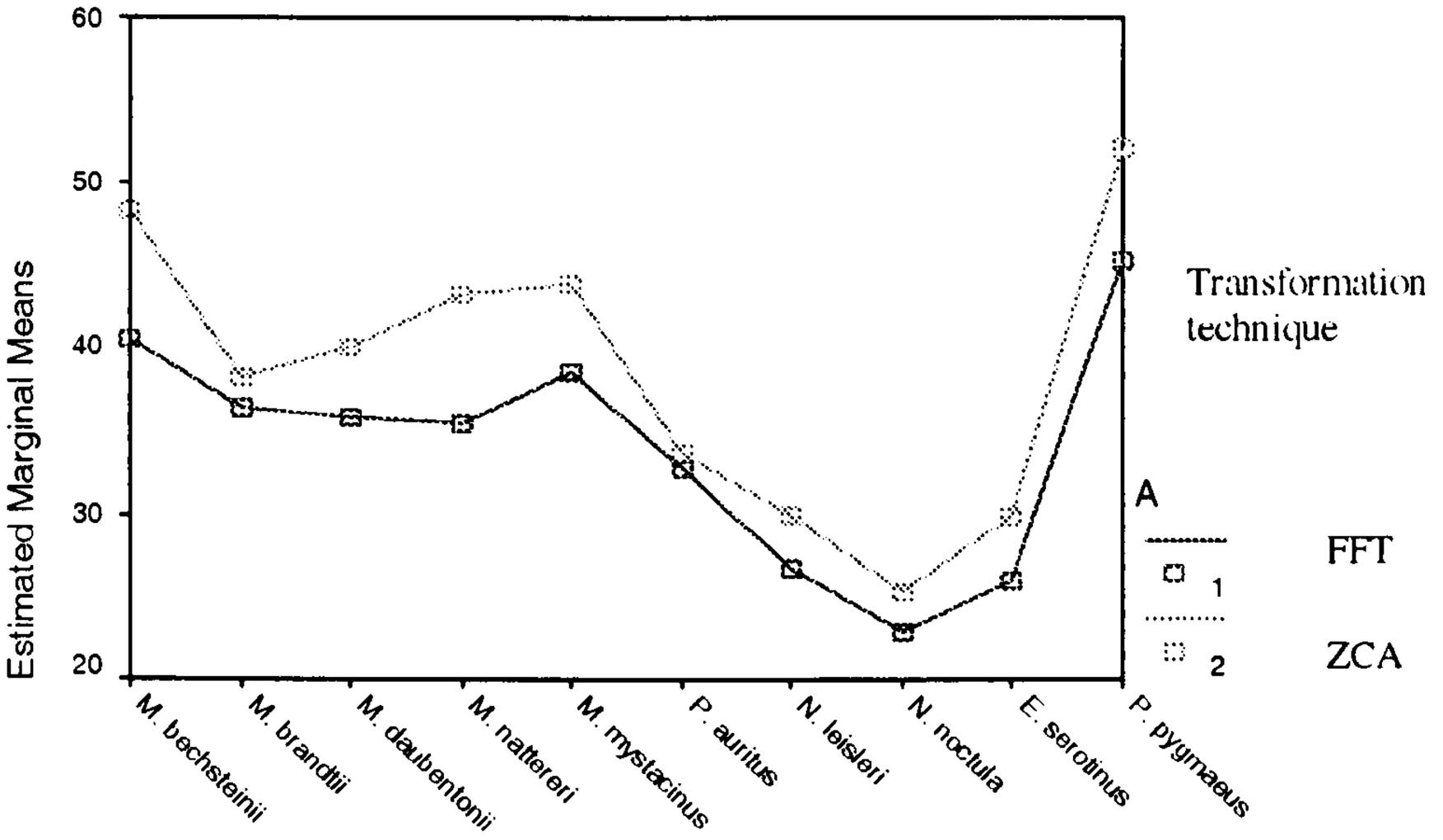


Fig. 2.18 Interaction between species and transformation method for end frequency.



Frequency of maximum energy

Species (ignoring all other variables) had a significant main effect on FmaxE indicating that the call parameter measures differed for different species (Table 2.6). Of the two variables frequency reduction and transformation techniques, only frequency reduction technique had a significant main effect on FmaxE (P<0.001) (Table 2.6). Significant interaction effects were seen for frequency reduction technique and species (P<0.001; Fig. 2.19), and for frequency reduction and transformation method used, but not for transformation method and species (Fig. 2.20). TE frequency reduction technique produced lower values for *P. auritus* and *N. leisleri* but higher values for all other species (Fig. 2.19). Figure 2.20 shows that for all species FFT transformation method generated lower or very similar values to those produced by the FD frequency reduction technique. A significant 3-way interaction between frequency reduction techniuque, transformation method used and species was also evident (P<0.001), indicating that the combined effect was different for different species. TE calls transformed by the FFT technique produced higher values compared to TE analysed by ZCA in *M. bechsteinii*, *M. brandtii*, *M. nattereri*, *P. auritus* and *E. serotinus*. ZCA transformation method produced lower values with FD calls in *M. brandtii*, *M. mystacinus* and *E.serotinus*, and higher values in all other species.

Table 2.6 Repeated measures ANOVA on frequency of maximum energy for within subject effects: frequency reduction and transformation technique, and between subject effect: species. Source =source of variation, df = degrees of freedom, * = interaction term.

Source	**df**	**F**	***P***
Species	9	125.3	<0.001
Frequency reduction	1	19.8	<0.001
Frequency reduction*species	9	3.4	0.001
Transformation	1	2.6	0.108
Transformation*species	9	1.7	0.097
Frequency reduction*transformation	1	8.5	0.004
Frequency reduction*transformation*species	9	3.8	<0.001

Fig. 2.19 Interaction between species and frequency reduction technique for frequency of maximum energy.

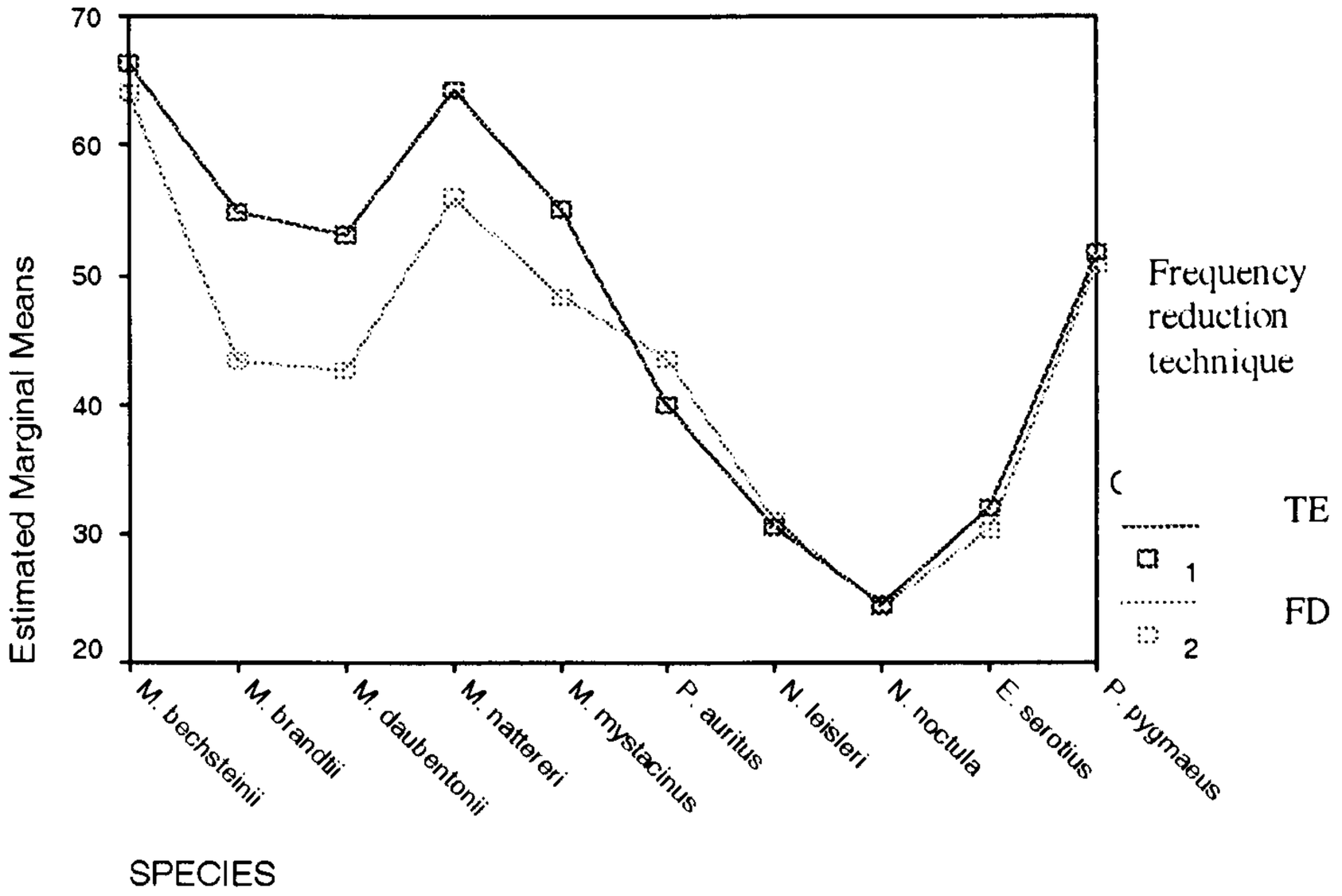
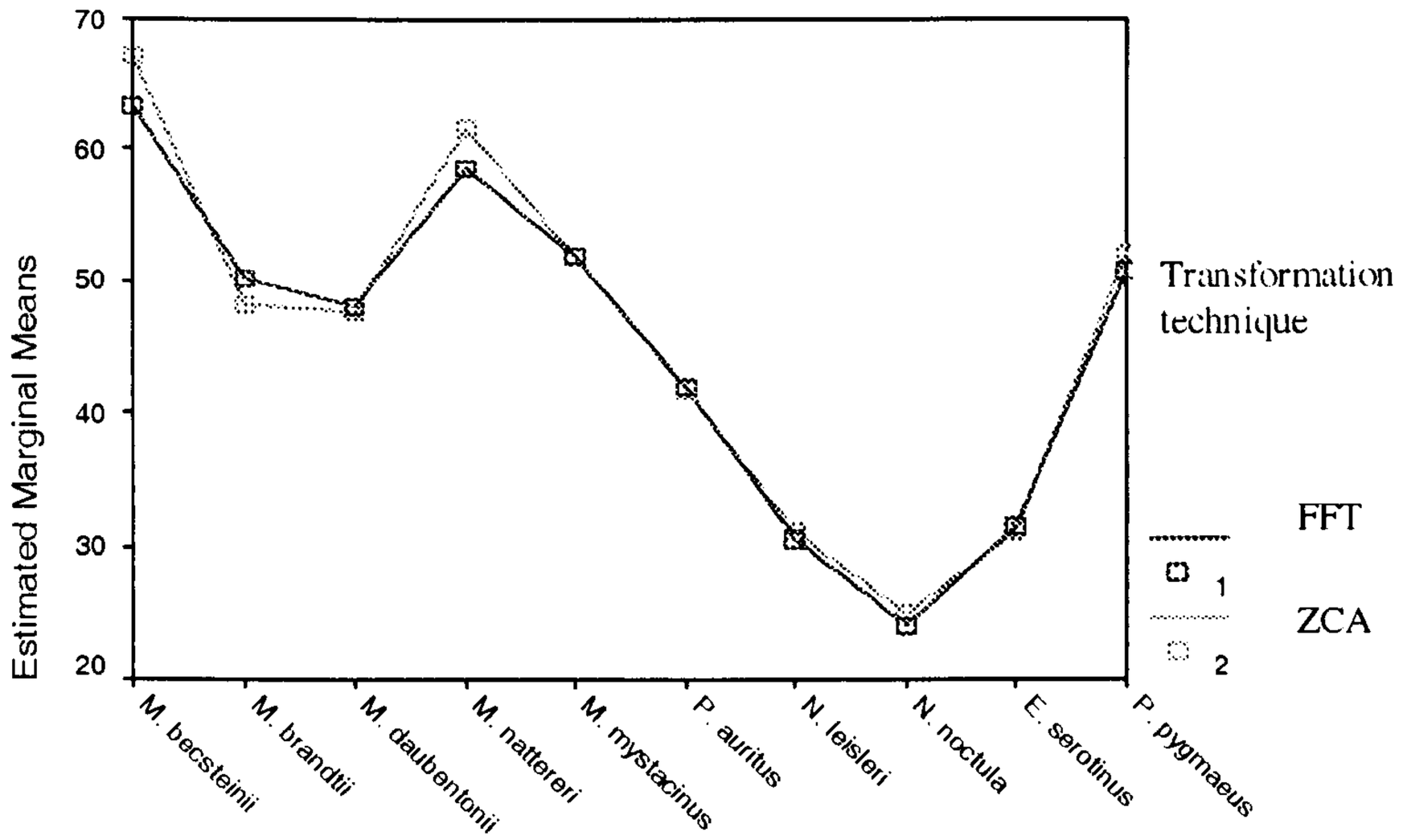


Fig. 2.20 Interaction between species and transformation method for frequency of maximum energy.



Central frequency

Species (ignoring all other variables) had a significant main effect on central frequency indicating that the call parameter measures differed for different species (Table 2.7). Both frequency reduction and transformation method had significant main effects on central frequency ($P<0.001$) (Table 2.7). The interaction between these two variables and species were also significant ($P<0.001$), indicating that the effect of frequency reduction or transformation method used was different depending on species (Figs 2.21 and 2.22). TE frequency reduction technique produced higher values compared to the FD technique for *M. nattereri* but lower values for all other species (Fig. 2.21). Figure 2.22 shows that the FFT transformation method generated higher values than the ZCA method for *M. nattereri*, *M. mystacinus*, *N. noctula* and *E. serotinus*, but lower values for the other species. The combined effect of frequency reduction technique and transformation method was significantly different for different species. When FFT transformation method analysed TE calls higher values were generated when compared to the ZCA transformation method analysing TE calls for *M. bechsteinii*, *M. brandtii*, *M. nattereri*, *M. daubentonii*, *M. mystacinus*, *P. auritus* and *E. serotinus*. ZCA transformation analysing FD calls produced higher values compared to FFT transformation method analysing FD calls in *M. bechsteinii*, *M. brandtii*, *M. daubentonii*, *M. nattereri*, *M. mystacinus*, *P. auritus*, *N. leisleri* and *P. pygmaeus*.

Table 2.7 Repeated measures ANOVA on central frequency for within subject effects: frequency reduction and transformation technique, and between subject effect: species. Source =source of variation, df = degrees of freedom, * = interaction term.

Source	df	F	*P*
Species	9	135.01	<0.001
Frequency reduction	1	25.4	<0.001
Frequency reduction*species	9	5.5	<0.001
Transformation	1	23.6	<0.001
Transformation*species	9	8.8	<0.001
Frequency reduction*transformation	1	91.04	<0.001
Frequency reduction*transformation*species	9	14	<0.001

Fig. 2.21 Interaction between species and frequency reduction technique for central frequency.

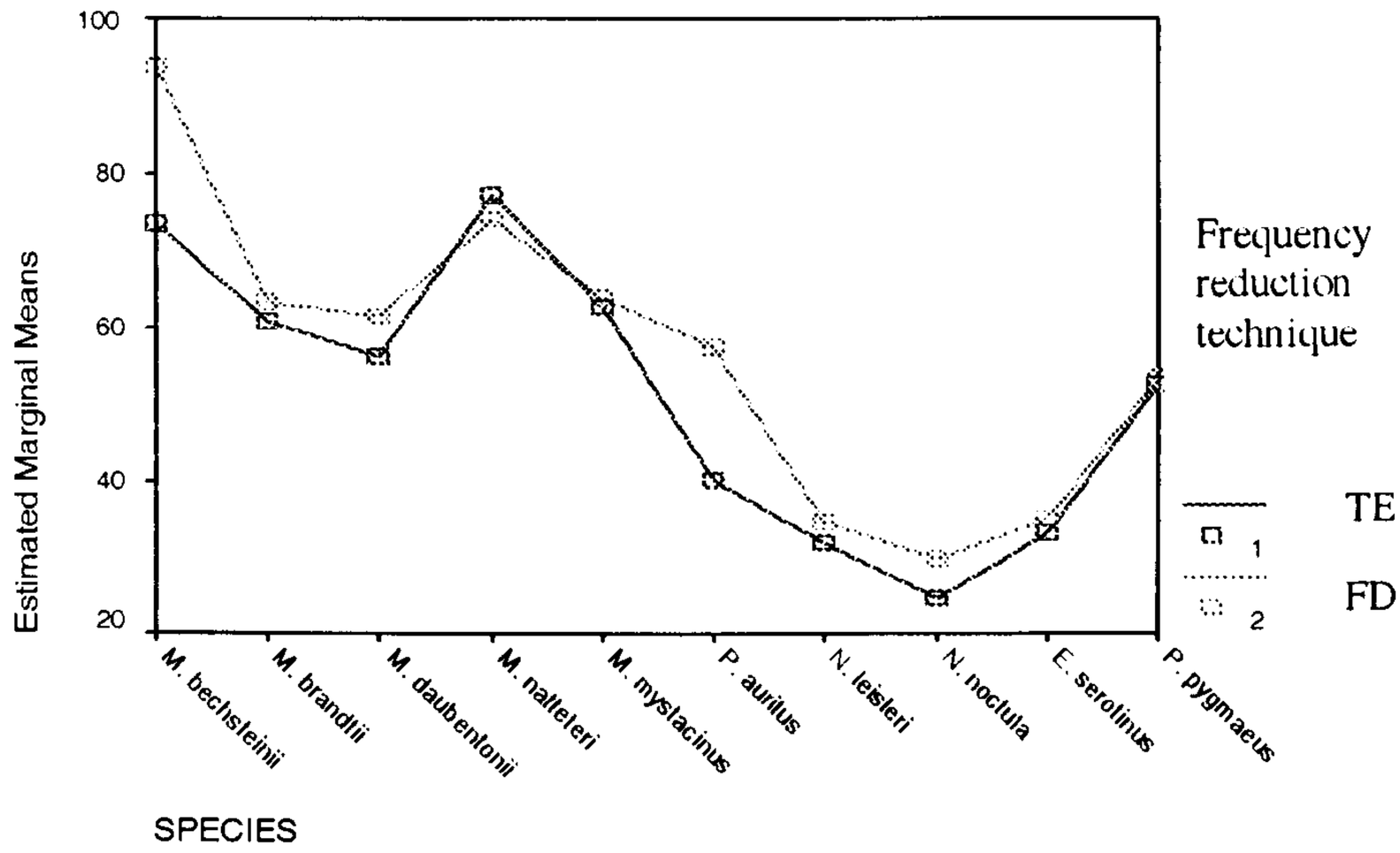
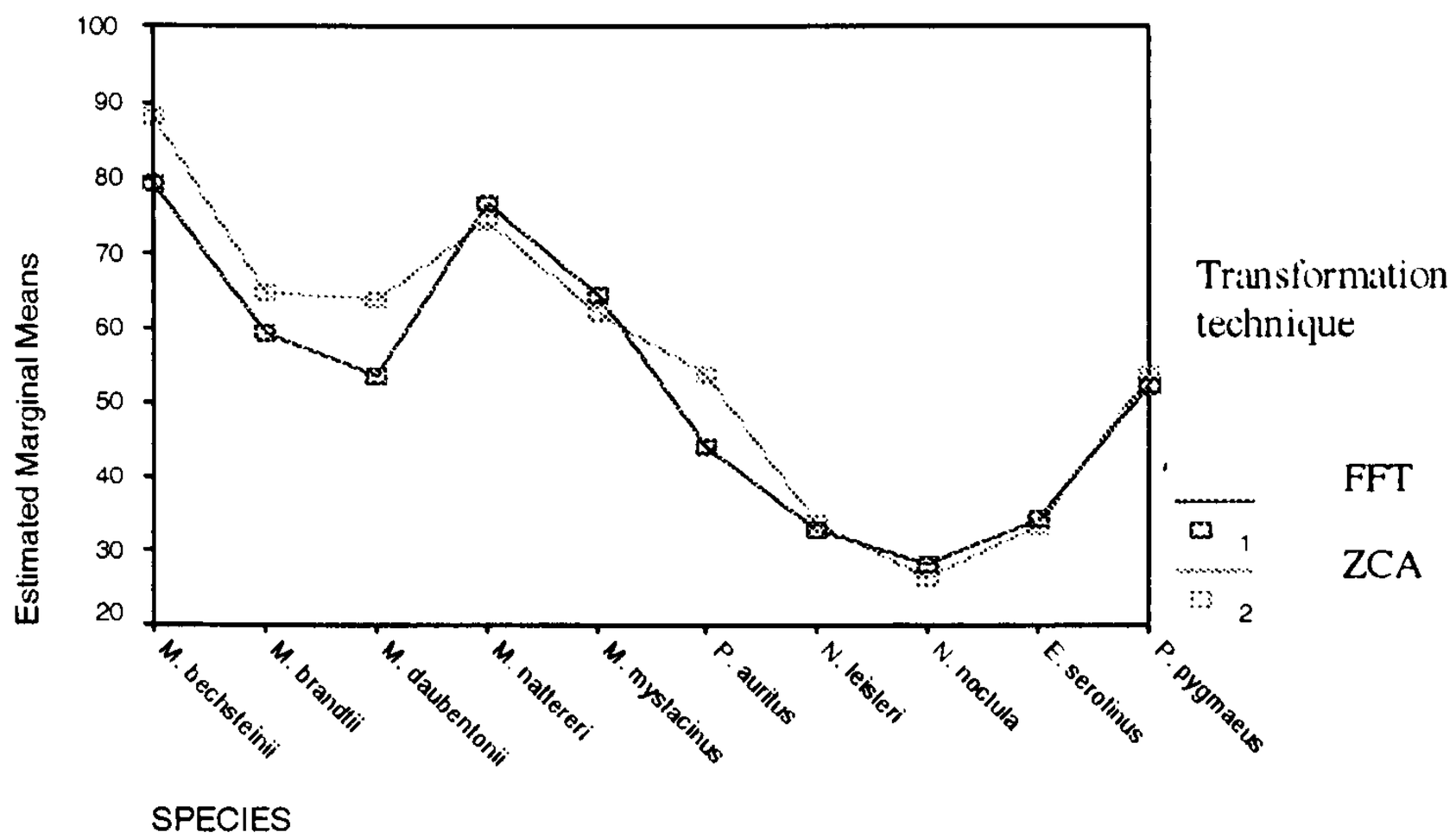


Fig. 2.22 Interaction between species and transformation method for central frequency.



The results of the doubly multivariate ANOVA testing all parameters together were significant across all main effects and interactions; Pillai's trace -2.330, ($F_{45,1315}$=25.5, P<0.001).

2.4 Discussion

The choice of frequency reduction and transformation techniques had significant overall effects on all call parameters. In addition these effects were different for different species.

For this study, it was important to keep the number of interfering variables to a minimum in all the stages of analysis, so that any differences seen could be explained by frequency reduction or transformation method. By using the same equipment I eliminated any technical variation within the recording, something that was not done by Fenton *et al.* (2001).

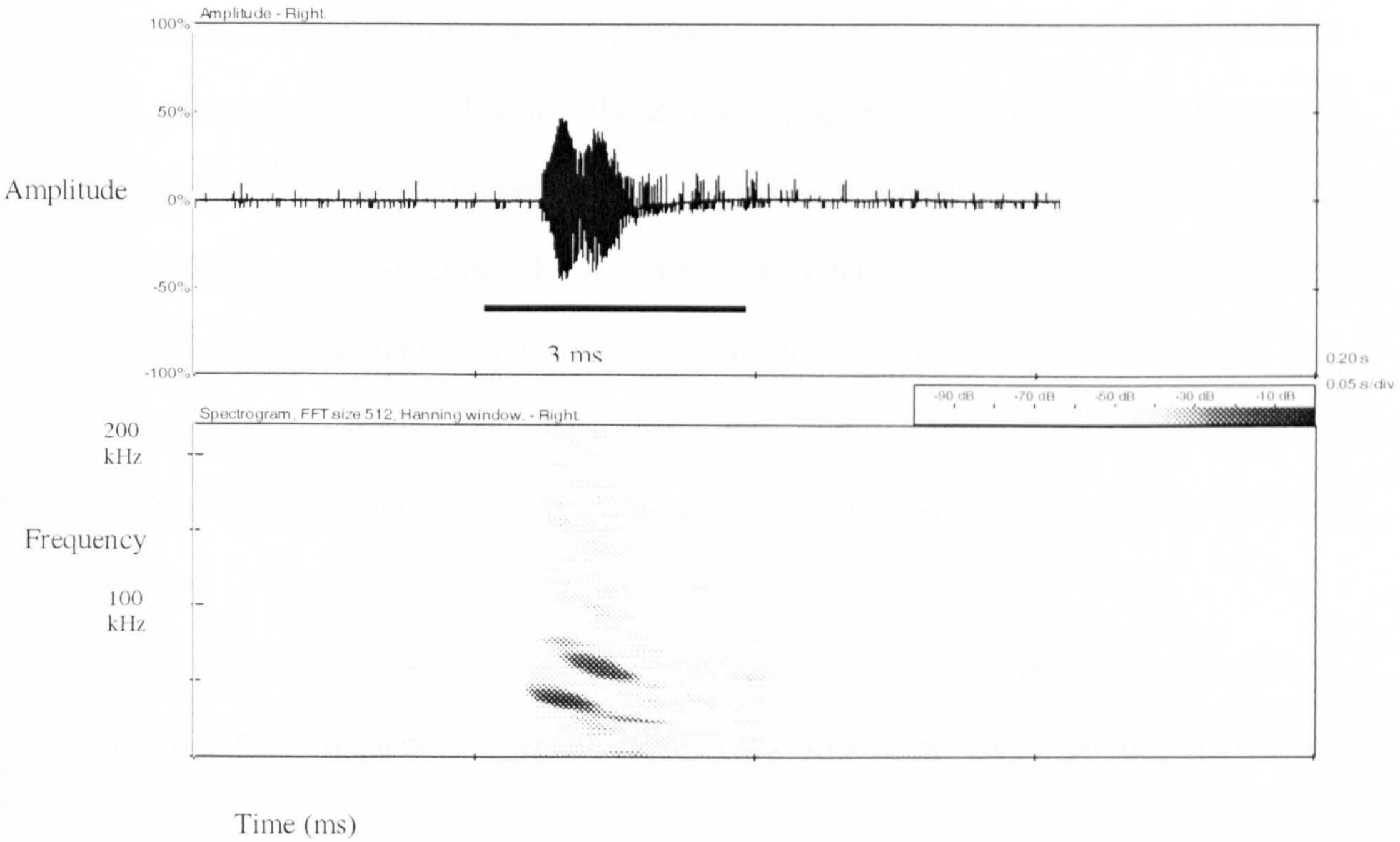
The TE/FFT results are slightly different to those published by Parsons & Jones (2000), especially for those species producing relatively short calls. This is probably due to recording method, and the fact that many of these species were recorded on release by hand, a technique which typically produces shorter calls (Parsons & Jones 2000). In general, most of the temporal and spectral measurements taken from the calls agree with those published in the literature (Ahlén 1981; Konstantinov & Makarov 1981; Zingg 1988; Jones & Rayner 1989; Kalko & Schnitzler 1989; Zingg 1990; Jones 1995; Waters *et al.* 1995; Vaughan *et al.* 1997; Britton & Jones 1999; Jensen & Miller 1999; Rydell *et al.* 1999). In contrast, the results from FD calls using FFT show some large deviations from the published data, particularly in F-start and F-end measurements. This can be attributed to the fact that only the strongest harmonics are measured, which can increase or decrease averages. Another possible reason for these differences is the analysis

method used. FFT is a technique that is heavily reliant on high information content in the incoming signal; with a FD signal this is not the case.

The TE/ZCA results agree with the TE/FFT results. One strange result is the higher value for *Plecotus auritus* start frequency. This is likely to be due to the fact that this species produces multi-harmonic signals (Fig. 2.23). As TE calls have all the harmonic information present, this can lead to misleading output when types of calls are analysed by ZCA. The other differences between this data set and the TE/FFT data set could be due to the different thresholding levels used by the different methods. The FD/ZCA data do not agree with the TE/FFT data, due to the low information content of the incoming signal.

To conclude, these results show that TE and FD calls recorded by the same equipment and analysed using comparative methods give significantly different descriptions of call parameters.

Fig. 2.23 Waveform and spectrogram of a multi-harmonic call emitted by *Plecotus auritus*.



The process of lowering the frequency of the calls produced by a bat for analysis, in this case by TE or FD, results in different descriptions of call parameters. In addition the methods used to analyse the spectral data also influence the call parameter measurements. The effects of both of these variables also vary with different species. Although all parameters were affected in some way, those most affected by both frequency reduction technique and transformation method were duration, start frequency and end frequency. All these parameters had higher values when analysed by the ZCA transformation method. Signal duration and end frequency were significantly greater for FD calls overall (i.e. for both methods), in agreement with Fenton *et al.* (2001). However in contrast to Fenton's findings, in this study start frequency from FD calls in general had higher values than TE calls. Parsons & Jones (2000) found that, in order of importance, end frequency, start frequency, centre frequency and frequency of maximum energy were crucial parameters for classification of calls to species.

In conclusion, FD calls with their low information content are not suitable for detailed call description. Due to the nature of ZCA, the calls of species that vary the harmonic content of their calls, or produce multi-harmonic calls, are poorly described by this method. This is because the zero-crossing system jumps between harmonics leading to a misleading output signal, especially when analysed spectrally (Parsons & Obrist 2003). Fenton (2000) showed that ZCA systems detect fewer bats compared to time-expansion systems, due to differences in sensitivity between the two systems. The results from the present study have shown that ZCA can be used with relatively good results if the incoming signal contains sufficient information as seen in the TE/ZCA results.

In agreement with Parsons *et al.* (2000), I conclude that the choice of technique to lower the frequency of echolocation calls should be based on the amount of

information needed from the transformed signal. If an accurate representation of the spectral content of the signal is required for identification purposes, the technique used should remove the lowest possible amount of information from the signal (Parsons *et al.* 2000).

2.5 Summary

- Frequency reduction techniques significantly affect call descriptions
- FD results in longer duration, higher start and higher end frequency parameters compared to time expansion. Generally FD calls have lower information content than TE calls
- The method of transforming calls into the frequency-time domain has a significant influence on the description of the calls
- The process of zero crossing is not capable of analysing multi-harmonic calls, leading to poor descriptions of those species that vary the harmonic content of their calls
- FD should not be used for detailed call description

Having determined that TE calls analysed by FFT result in more accurate representations of a bat call than FD calls analysed using ZCA, in the next Chapter I examine how different the classification rates of the calls produced by these two methods are.

CHAPTER 3

ACOUSTIC SPECIES IDENTIFICATION OF UK BATS (II) – IDENTIFICATION USING DISCRIMINANT FUNCTION ANALYSIS (DFA) AND ARTIFICIAL NEURAL NETWORKS (ANN)

3.1 Introduction

Accurate identification of bat species from their echolocation calls has until recently been difficult and previous methods could often only identify bats to genus or by grouping them by foraging strategy (Sherwin *et al.*2000). Although some bat species are easily discriminated due to characteristic call design (*Rhinolophus* species), others such as the *Myotis* species are difficult to separate due to similarities in call design used by members of this genus, perhaps as a result of phylogenetic constraints or convergence (Parsons & Jones 2000).

Much of the published quantitative work on species identification of bats has used multivariate statistics, notably discriminant function analysis (DFA), which has resulted in varying levels of success (Zingg 1990; Neefus & Krusic 1995; Obrist 1995; Parsons 1997, Vaughan *et al.*1997; Parsons & Jones 2000; Russo & Jones 2002). Recently artificial neural networks (ANNs), another method of multivariate analysis of call parameters, have been used to separate calls made by different species. ANNs have been used in many areas of bioacoustics, for example target classification from echoes by cetaceans (Au 1994; Au *et al.* 1995), classifying the vocalisations of marine mammals (Murray *et al.* 1998; Deecke *et al.* 1999), species identification of fish schools (Haralabous & Geogakarakos 1996) and identification of bat species from echolocation calls (Wotton & Jenison 1997; Parsons & Jones 2000; Parsons 2001). ANNs have been

shown to achieve higher rates of correct classification to species than DFA (Parsons & Jones 2000).

3.1.1. Discriminant function analysis

DFA is used to build a predictive model of group membership based on observed characteristics of each case (Ryan 1994). The analysis computes a new variable (Z), which is a linear or quadratic function of the measured variables (Sokal & Rohlf 1981; Parsons & Obrist 2003). When the variables from each individual are plotted against one another, the linear or quadratic function represents the equation of a line that best discriminates between groups (Parsons & Obrist 2003). The output gives an apparent percentage error, which is the percentage of misclassified observations. This percentage error is optimistic, as the data classified are the same data used to build the classification functions. The percentage error can be made more realistic by using cross-validation, which omits each observation at a time, re-calculating classifications using the remaining data and then classifying the omitted observations (Ryan 1994).

3.1.2. Artificial neural networks

ANNs are systems designed to model the way in which the human brain performs a particular task (Haykin 1999). The human brain learns through experience, which is built up over time. The definition of a neural network given by Haykin (1999) is as follows:

> *A neural network is a massively parallel distributed processor made up of simple processing units, which has a natural propensity for strong experiential knowledge and making it available for use. It resembles the brain in two respects:*

1. *Knowledge is acquired by the network from its environment through a learning process.*
2. *Interneuron connection strengths, known as synaptic weights, are used to store the acquired knowledge.*

An ANN contains networks of simple processing units called neurons. These neurons are arranged so that each neuron in one layer is connected to every neuron in the preceding layer (Terry *et al.* 2002). The learning process is achieved by modifying synaptic weights between units of neurons in an ordered fashion to attain a desired objective. This adaptivity of ANNs means that if variables change with time the ANN can be designed to change its synaptic weights (Haykin 1999).

In this study, back-propagation networks were used. These are a form of multilayer perceptron, which can be 'taught' to recognise patterns so that they have the ability to classify previously unseen data (Parsons *et al.* 2000). A perceptron is the simplest form of neural network used for the classification of patterns separated linearly (Haykin 1999). Back-propagation learning consists of two passes through the different layers of the network – a forward and a backward pass. The forward pass goes through each layer and an output is achieved; during this process the synaptic weights are fixed. In the backward pass the weights are adjusted in order to minimise the error (Haykin 1999; Parsons *et al.* 2000). This means that unlike in DFA, the first result is not necessarily the final one. The ability of ANNs to generalise (produce reasonable outputs for inputs not encountered during training) utilise an error-minimisation algorithm, and solve non-linear classifications sets them apart from DFA (Haykin 1999; Parsons & Obrist 2003). Published data (Parsons & Jones 2000) suggest that results from the ANN will be better than from the DFA for the identification of bats from time expanded

echolocation calls. This is partly due to the error minimisation mentioned earlier, and also because ANNs have the ability to partition the workload. In a multi-layered network, sub networks can handle small problems (e.g. separating calls of *B. barbastellus* from those of *N. noctula*) and others can tackle more complex problems (e.g. separating calls of *M. bechsteinii* from those of *M. nattereri*). Workload partitioning is not possible with DFA.

3.1.3. Aims of this chapter

In Chapter 2 I showed that FD calls analysed using an FFT method resulted in descriptions that were not representative, and although the results from TE using ZCA were in agreement with TE/FFT results, it is a technique that will not be used in practical applications, so both these data sets have been omitted from this analysis. The two systems commonly used, TE calls transformed by FFT and FD calls transformed by ZCA, were analysed using both DFA and ANNs and the results compared. Classification was made to both genus and species.

Although FD calls poorly describe bat calls compared to TE, if species of bat could be grouped through classification with a reported degree of accuracy from FD calls, then this system could prove useful where an accurate survey of activity or diversity is needed. Using FD calls to identify bat species with a degree of accuracy would be a good, relatively cheap, practical system, and because no recording time is lost time expanding the signals, developing a real-time system of identification could be a possibility.

3.2 Methods

3.2.1. Recording of echolocation calls

The recording methods and calls used in this analysis are the same as those in Chapter 2. The data were tested for multivariate normality by using Box's M test before DFA was performed using statistical software (Minitab v13, Ryan & Joiner 1994). Most of the variables measured from both TE and FD echolocation calls did not conform to the multivariate normality distribution (Box's M test, $F=10.07$, $P<0.0001$). However, DFA is relatively robust to deviations from normality (Dillon & Goldstein 1984; Parsons & Jones 2000). The covariance matrices were heterogeneous, and data transformations did not reduce this, nor did they reduce deviations from normality. Therefore, quadratic discriminant functions were calculated and cross validation was used in all DFAs (Parsons & Jones 2000).

Due to the ease of separating *Rhinolophus ferrumequinum* and *R. hipposideros* from each other and from other species, data on *Rhinolophus* species have been omitted from Tables 3.1 and 3.2. They are included in the genus analysis for comparison between groups.

3.2.2. Design and training of the ANN

Multilayer perceptrons were trained using a back-propagation algorithm with momentum (Haykin 1999; Rumelhart *et al.* 1986; Parsons & Jones 2000), epoch training and adaptive learning (Vogl *et al.* 1988; Parsons & Jones 2000) using the neural network toolbox (toolbox version 3.01) of Matlab v 6. The general network architecture and training methods were similar to those used by Parsons & Jones (2000). Two approaches were tried. A number of network architectures were trained depending on the classification task required. The first approach was a single large network

classifying all calls to species. The second was a network classifying calls to genus and other networks for multi-species genera that take the genus result and further classify calls to species. The latter approach is termed the genus-specific hierarchical approach.

The inputs to the networks were the five temporal and spectral variables described in Chapter 2, and the outputs were the 12 species emitting the calls to be classified. Either one or two hidden layers were used. The number of neurones in each layer was varied between five and 20, and the momentum constant was varied between 0.1 and 0.9 in steps of 0.1. The most suitable network architecture was defined as that giving the highest correct identification rate. The networks were trained using 50% of the input data. To standardise the scales of measurement for each parameter, the training and testing data were converted to centred reduced variables by the relationship:

$$Z_P = \frac{X_P - \overline{X}}{\sigma_X}$$

Z_P: standardised variable, X_P: original values, $\overline{X}$ and σ_X: mean and standard deviation of the variable.

The performance of the network during training was represented by the root-mean-squared (RMS) error of observed versus expected outputs. In order to achieve reasonable performance, the training algorithm was repeated up to five thousand epochs, or until the RMS error was reduced to an arbitrary level (0.05). After training, the remaining 50% of the input data was used to test the networks independently. The five architectures producing the highest overall classification rate were rerun 30 times, each time using different initial random weights and biases for each neurone, to ensure that the highest classification rate had been achieved.

3.3 Results

3.3.1. Discriminant function analysis results

Discriminant function analysis of the parameters measured from 273 time expanded calls transformed using FFT (TE/FFT) from 10 species gave an overall correct classification rate of 76% (Fig. 3.1). The highest correct discrimination rate was achieved for *Pipistrellus pygmaeus*; 97% of recorded calls were correctly identified. Five species (*N. leisleri*, *E. serotinus*, *M. daubentonii*, *M. mystacinus* and *M. nattereri*) had over 70% of their recorded calls identified correctly. The remaining four species had less than 70% of their calls identified correctly. The calls of *Plecotus auritus* were the most difficult to classify, with only 55% correctly identified.

DFA of the parameters measured from the 273 frequency divided calls transformed using ZCA (FD/ZCA), gave an overall correct classification rate of 52% for all species (Fig. 3.1). Again the highest correct classification rate was achieved for *Pipistrellus pygmaeus*, with 97% of recorded calls correctly identified. Two species (*N. noctula* and *E. serotinus*) both had over 80% of their calls identified correctly. *M. mystacinus* had over 60% of its recorded calls correctly identified. The remaining six species had correct classification rates below 50%. *N. leisleri* was the most difficult to classify, with only 9% of calls correctly identified. The majority of *N. leisleri* calls were miss-classified as *E. serotinus*. Overall in Fig 3.1 TE/FFT provided better results in eight out of the 11 cases, but FD/ZCA was better for classifying *E. serotinus* and *N. noctula*. Both TE/FFT and FD/ZCA achieved the same classification rate for *P. pygmaeus*.

DFA was also used to classify calls to genus level (Fig. 3.2). For TE/FFT calls, the overall correct classification rate for genus was 92%. A correct classification rate of 83% was achieved for *Nyctalus* species and 98% for *Myotis* species. DFA was used on

the FD/ZCA data to classify calls to genus level (Fig. 3.2) with an overall correct classification rate of 67%. This produced correct identification rates of 69% for *Myotis* and 20% for *Nyctalus*. The discriminant function results are shown in Tables 3.1 and 3.2. Overall in Fig 3.2 TE/FFT was better at classifying to genus than FD/ZCA in four out of the seven cases. FD/ZCA was, however, better at genus classification for *E. serotinus*. The genus Rhinolophus achieved 100% classification rates for both TE/FFT and FD/ZCA as expected.

Fig. 3.1 Correct species identification rates from discriminant function analysis.

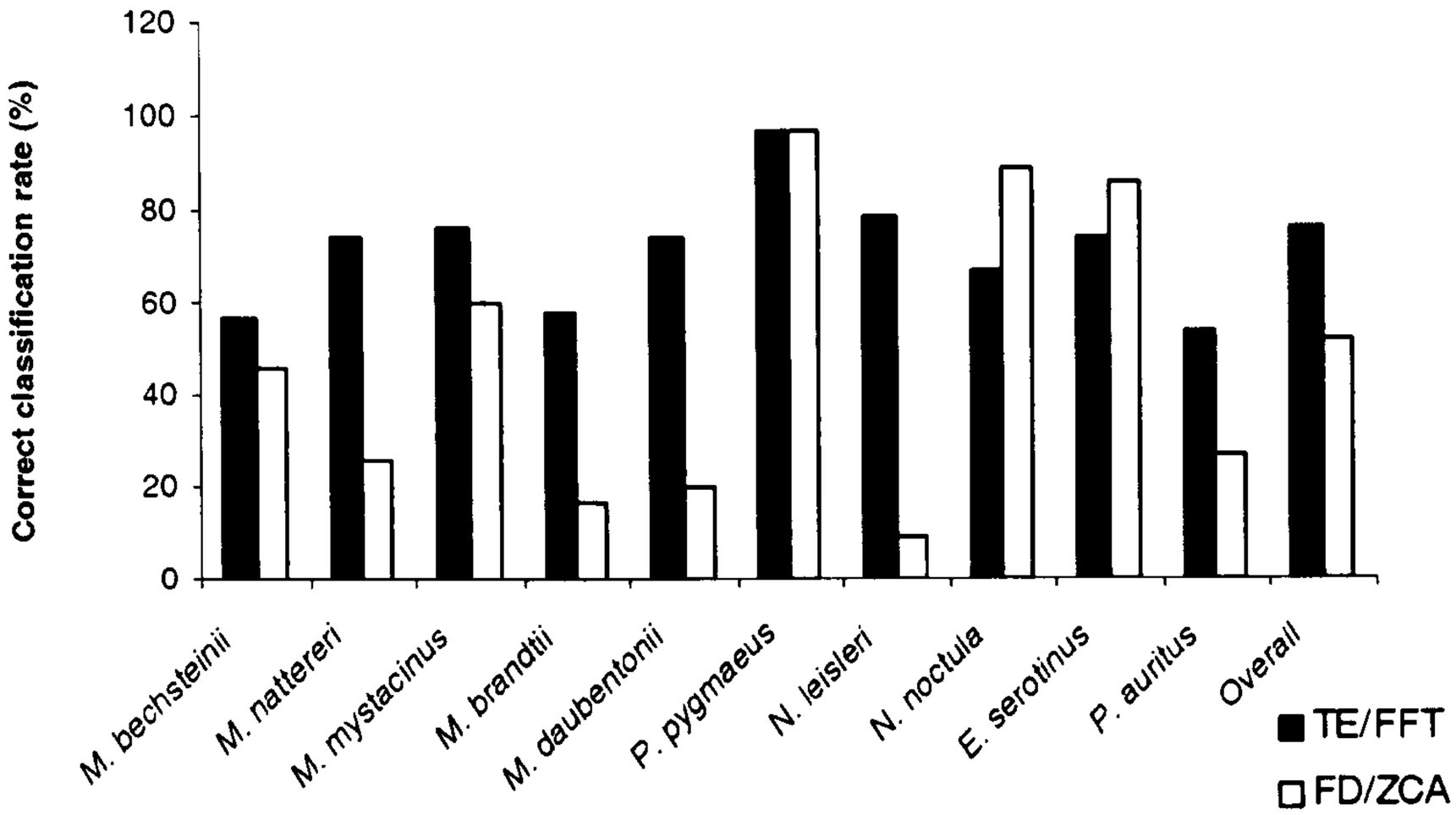


Fig. 3.2 Correct genus identification rates from discriminant function analysis.

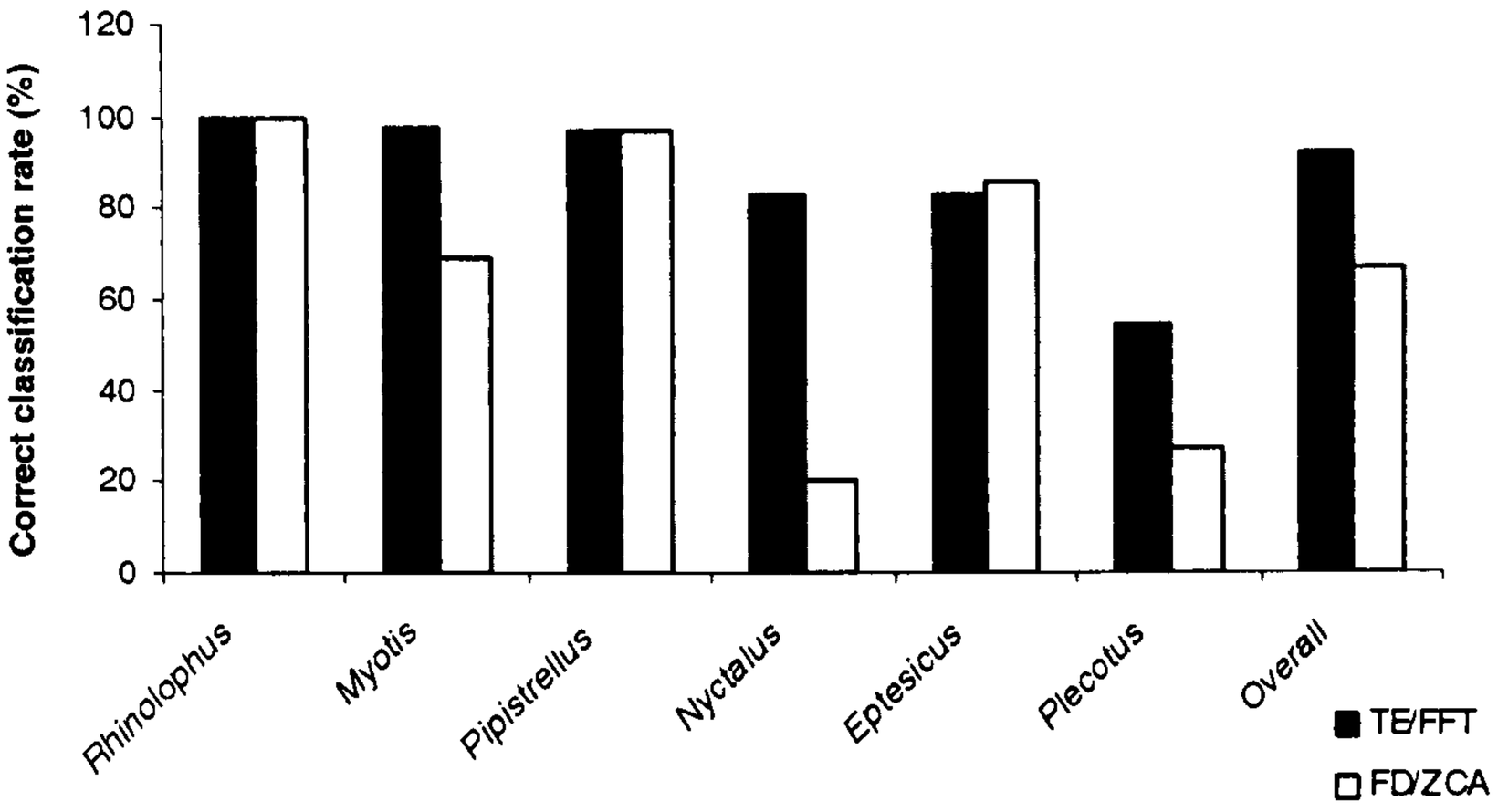


Table 3.1 Results from the discriminant analysis for TE/FFT data.

	True Group									
Classified as	*M. bechsteinii*	*M. brandtii*	*M. daubentonii*	*M. mystacinus*	*M. nattereri*	*N. leisleri*	*N. noctula*	*P. pygmaeus*	*E. serotinus*	*P. auritus*
M. bechsteinii	20	0	2	3	6	0	0	1	0	2
M. brandtii	2	7	1	1	0	0	0	0	0	0
M. daubentonii	2	2	26	2	0	0	0	0	0	1
M. mystacinus	5	1	5	19	0	0	0	0	0	0
M. nattereri	6	2	0	0	17	0	0	0	0	0
N. leisleri	0	0	0	0	0	26	5	0	4	0
N. noctula	0	0	0	0	0	1	18	0	4	0
P. pygmaeus	0	0	0	0	0	0	0	36	0	0
E. serotinus	0	0	0	0	0	6	4	0	26	2
P. auritus	0	0	1	0	0	0	0	0	1	6
Total N	35	12	35	25	23	33	27	37	35	11
% Correct	57	58	74	76	74	79	67	97	74	55

Table 3.2 Results from the discriminant analysis for FD/ZCA data.

	True Group									
Classified as	*M. bechsteinii*	*M. brandtii*	*M. daubentonii*	*M. mystacinus*	*M. nattereri*	*N. leisleri*	*N. noctula*	*P. pygmaeus*	*E. serotinus*	*P. auritus*
M. bechsteinii	16	2	2	0	6	1	0	0	0	2
M. brandtii	9	2	11	3	1	0	0	0	0	1
M. daubentonii	3	3	7	2	3	0	0	0	0	4
M. mystacinus	1	4	0	15	1	0	0	1	0	1
M. nattereri	6	0	5	2	6	0	0	0	0	0
N. leisleri	0	0	2	0	1	3	0	0	2	0
N. noctula	0	0	0	0	0	2	24	0	1	0
P. pygmaeus	0	0	0	0	0	0	0	36	0	0
E. serotinus	0	0	0	0	3	26	3	0	30	0
P. auritus	0	1	8	3	2	1	0	0	2	3
Total *N*	35	12	35	25	23	33	27	37	35	11
% Correct	46	17	20	60	26	9	89	97	86	27

3.3.2. Artificial neural network results

For TE/FFT data, the ANN considering all species (overall network) produced an overall correct classification rate of 81% (Fig. 3.3). Of the five *Myotis* species, three achieved correct classification rates above 70%, with *M. daubentonii* obtaining perfect classification but *M. brandtii* getting 0% correct. Both *N. noctula* and *P. pygmaeus* achieved 100% classification rates (Fig. 3.3). The ANN classifying to genus gave an overall classification rate of 97% (Fig. 3.4). 100% correct classification rates to genus were achieved for all species except *E. serotinus* (82%) and *P. auritus* (60%).

For FD/ZCA data, an overall correct classification rate of 70% was achieved. Classification rates varied between 17% and 92% for the *Myotis* species. Classification to genus produced an overall correct classification rate of 91%. The classification rates to genus for all species were above 80%. As expected, the network classifying to genus produced higher results for classification than the overall network. The TE/FFT results reached 100% correct classification rates for most genera (Fig. 3.4).

Overall in Fig 3.3, TE/FFT achieved better results in seven out if the 11 cases. FD/ZCA achieved better results over TE/FFT for *E. serotinus*, *M. mystacinus* and *M. brandtii*. In Fig. 3.4, TE/FFT achieved better classification rates than FD/ZCA for four out of the seven cases, matching the result from FD/ZCA in two cases. FD/ZCA achieved better results than TE/FFT for *E. serotinus*.

Fig. 3.3 Correct species identification rates from the overall ANN

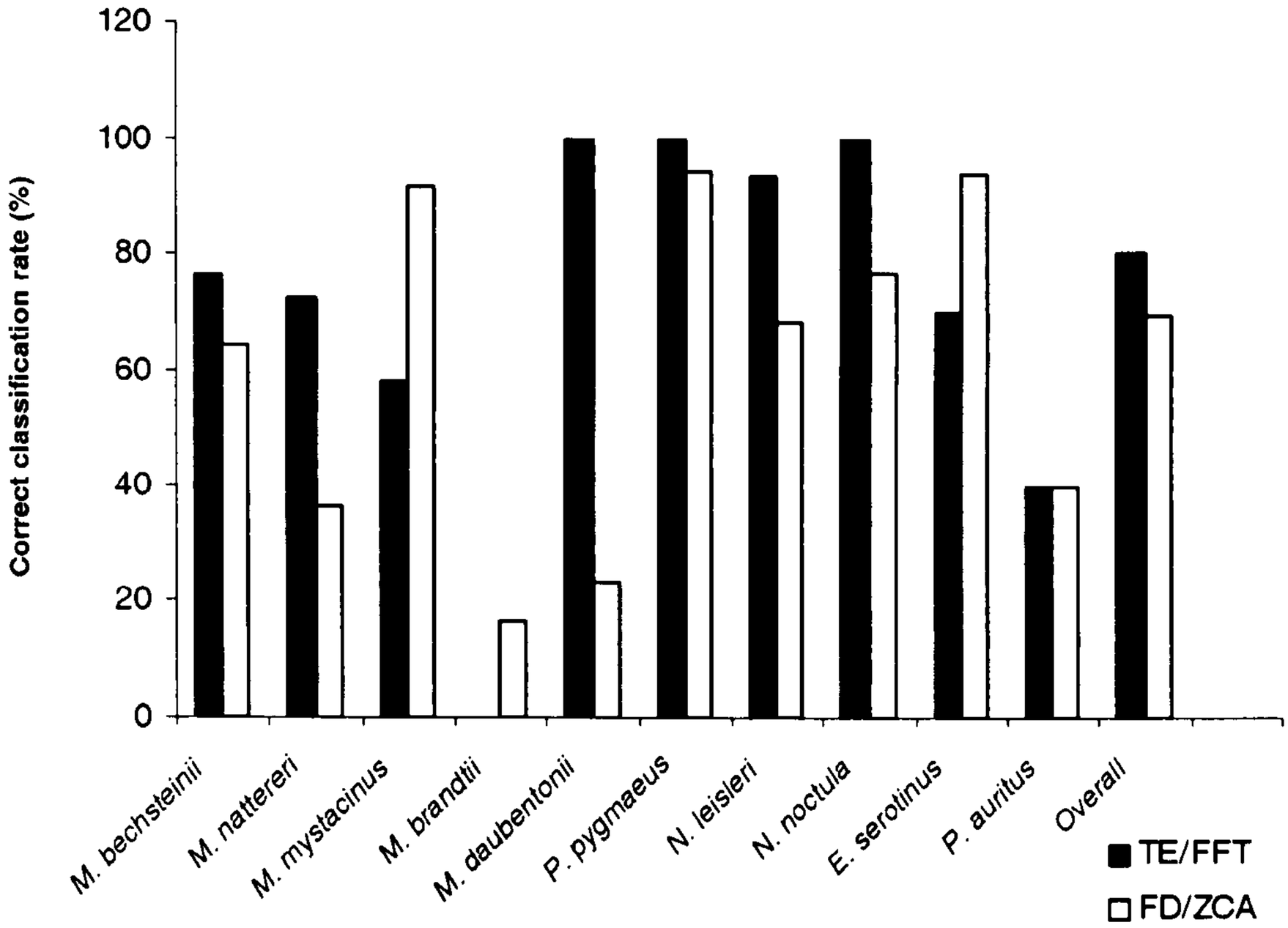
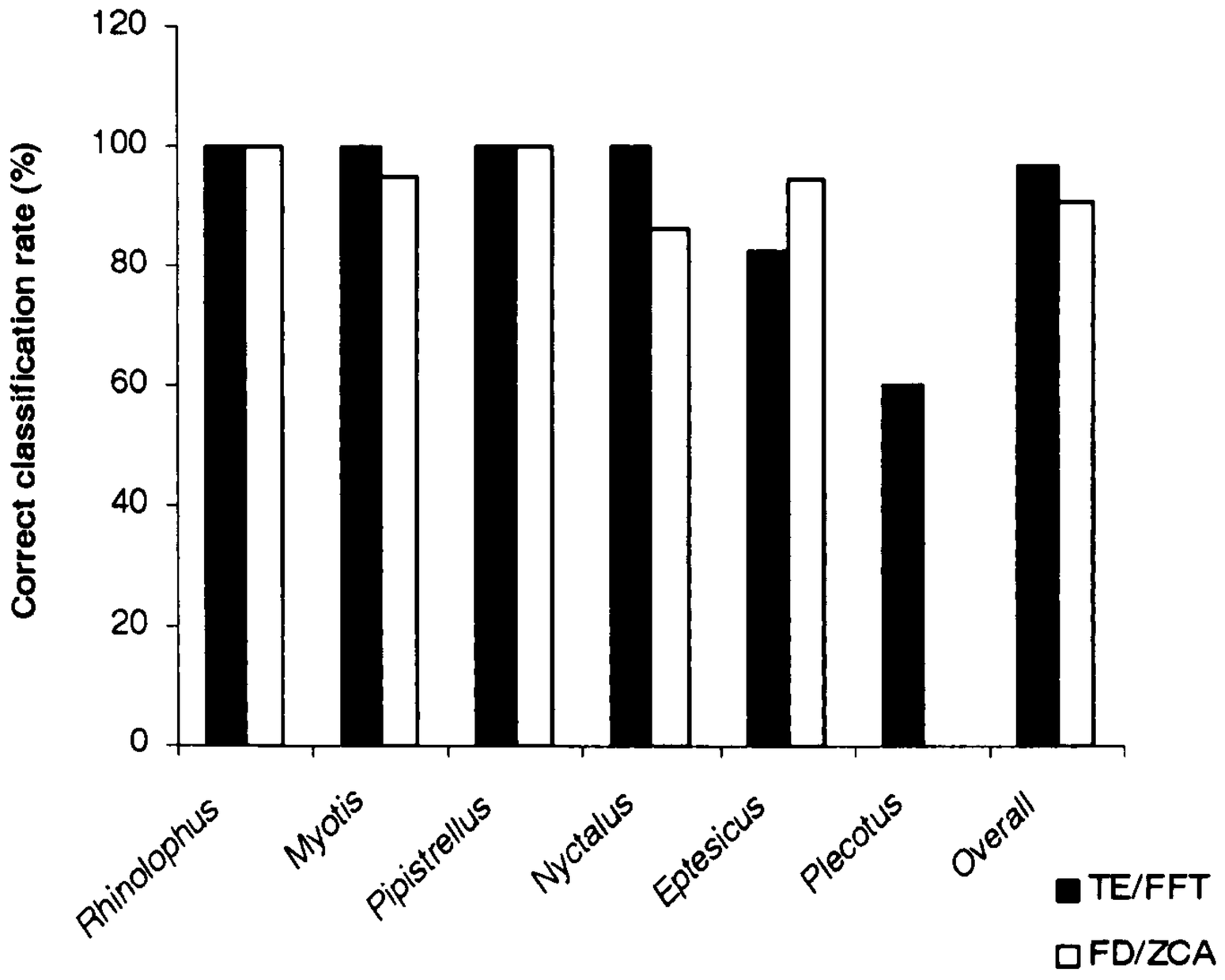


Fig. 3.4 Correct classification to genus by the ANN.



Genus-specific hierarchical networks (those that classify specific groups e.g. only *Myotis*) were also trained to see if species classification would improve with this

network architecture. Because of the difficulty in separating *Myotis* species, the results for this genus are presented in Table 3.3. With the genus-specific network design, TE/FFT produced higher species classification rates than FD/ZCA. The genus-specific networks produced better classification rates than the overall network considering all species. TE/FFT results obtaining higher rates were better than those using FD/ZCA. Figures 3.5 and 3.6 show the comparison between the correct classification rates produced by ANN and DFA.

Table 3.3 Correct classification rates (%) for the hierarchical network for *Myotis* species.

Species		***Myotis***	
		TE/FFT	FD/ZCA
Overall	*n*	81	52
M. bechsteinii	17	82	59
M. nattereri	11	91	54
M. mystacinus	12	67	75
M. brandtii	6	50	0
M. daubentonii	17	94	47

The hierarchical network results also proved to be better than the overall network in classifying species for both *Nyctalus* and *Rhinolophus* specific network architectures. *N. leisleri* achieved 94 and 100% correct classification rates for TE/FFT and FD/ZCA respectively. *N. noctula* obtained 100% correct classification rate.

Fig. 3.5 A comparison of correct identification rates achieved by ANNs using genus-specific hierarchical analysis and DFA on the TE/FFT data.

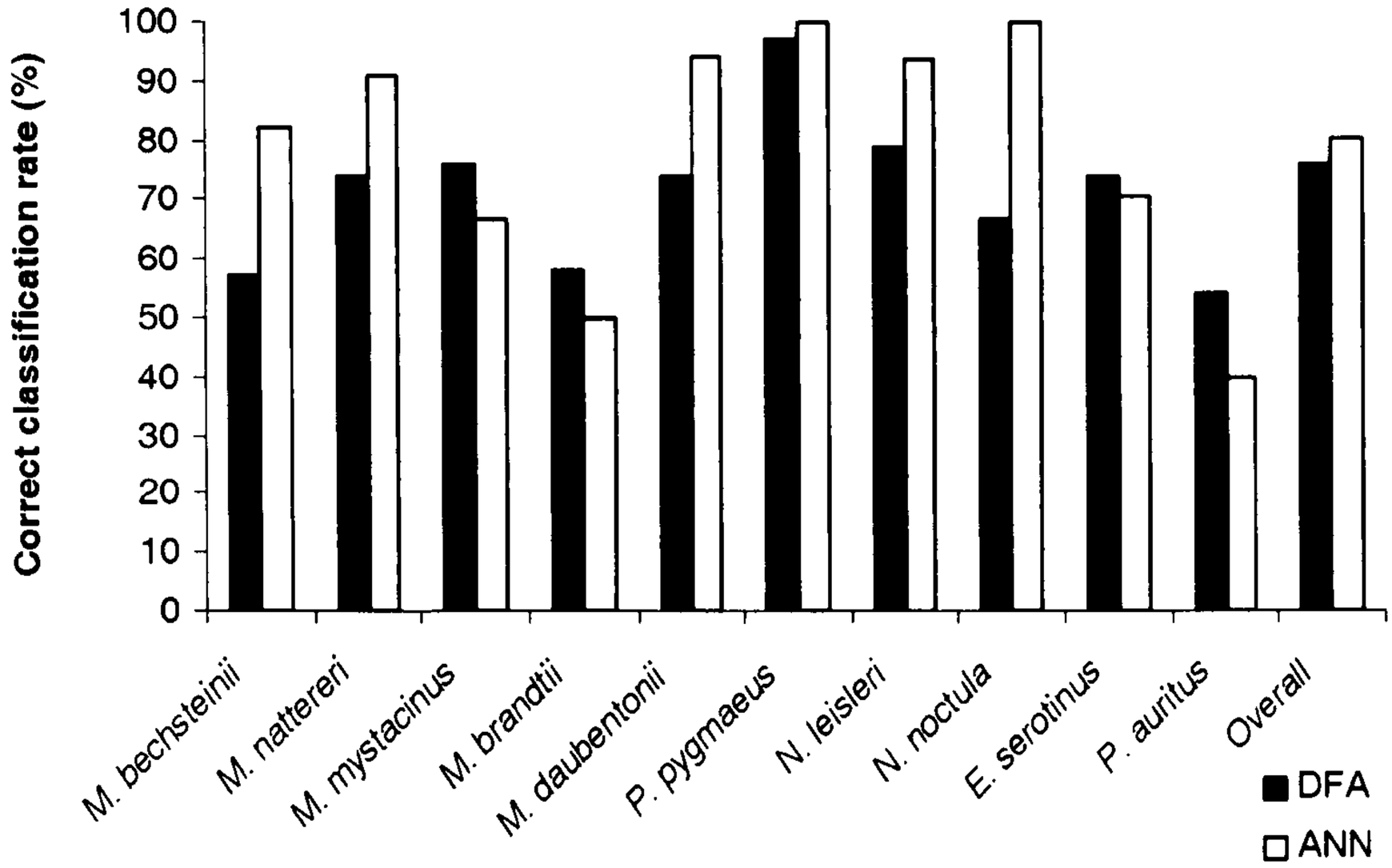
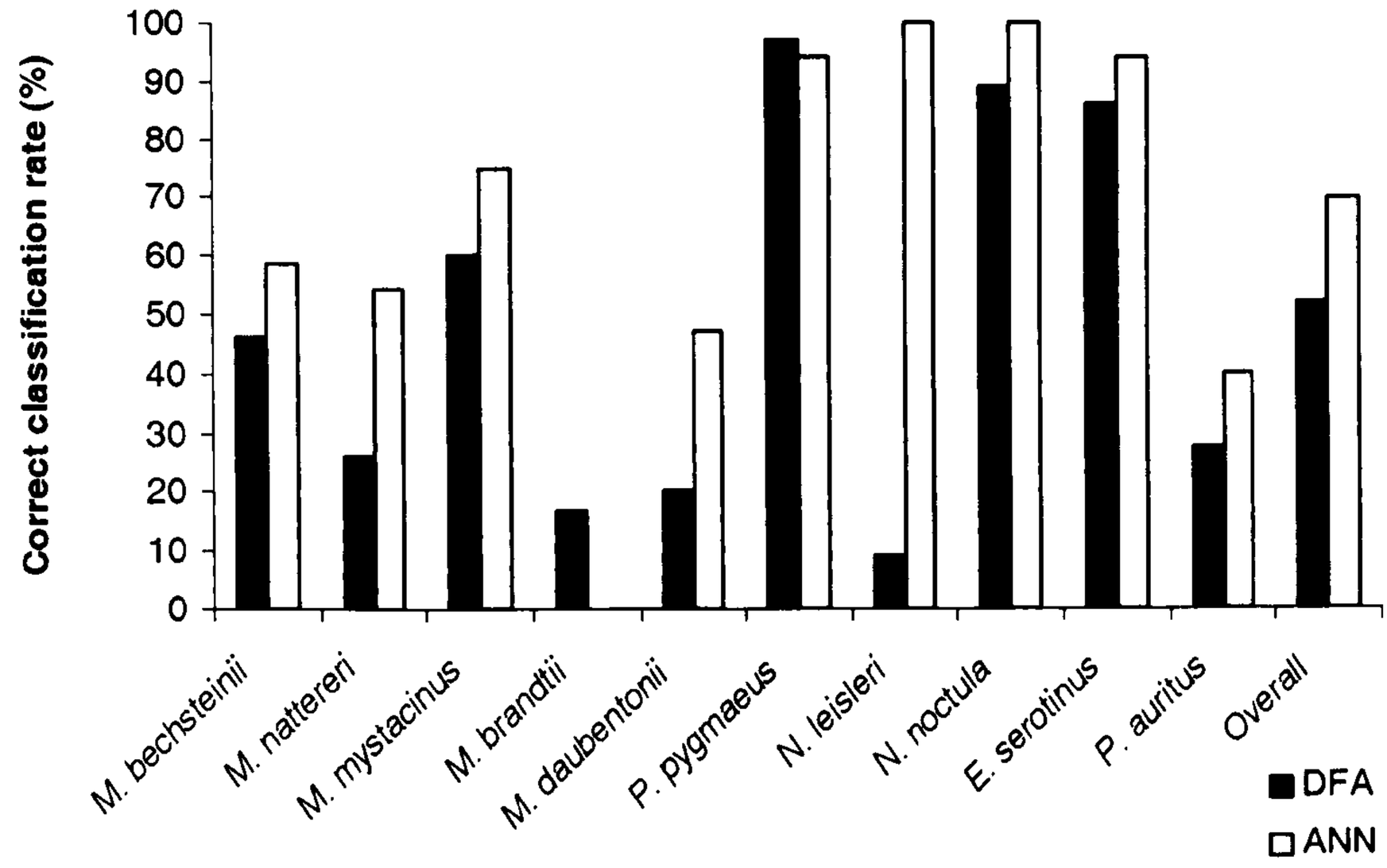


Fig. 3.6 A comparison of correct identification rates achieved by ANNs using genus specific hierarchical analysis and DFA on the FD/ZCA data.



3.3.3. **Myotis** *data*

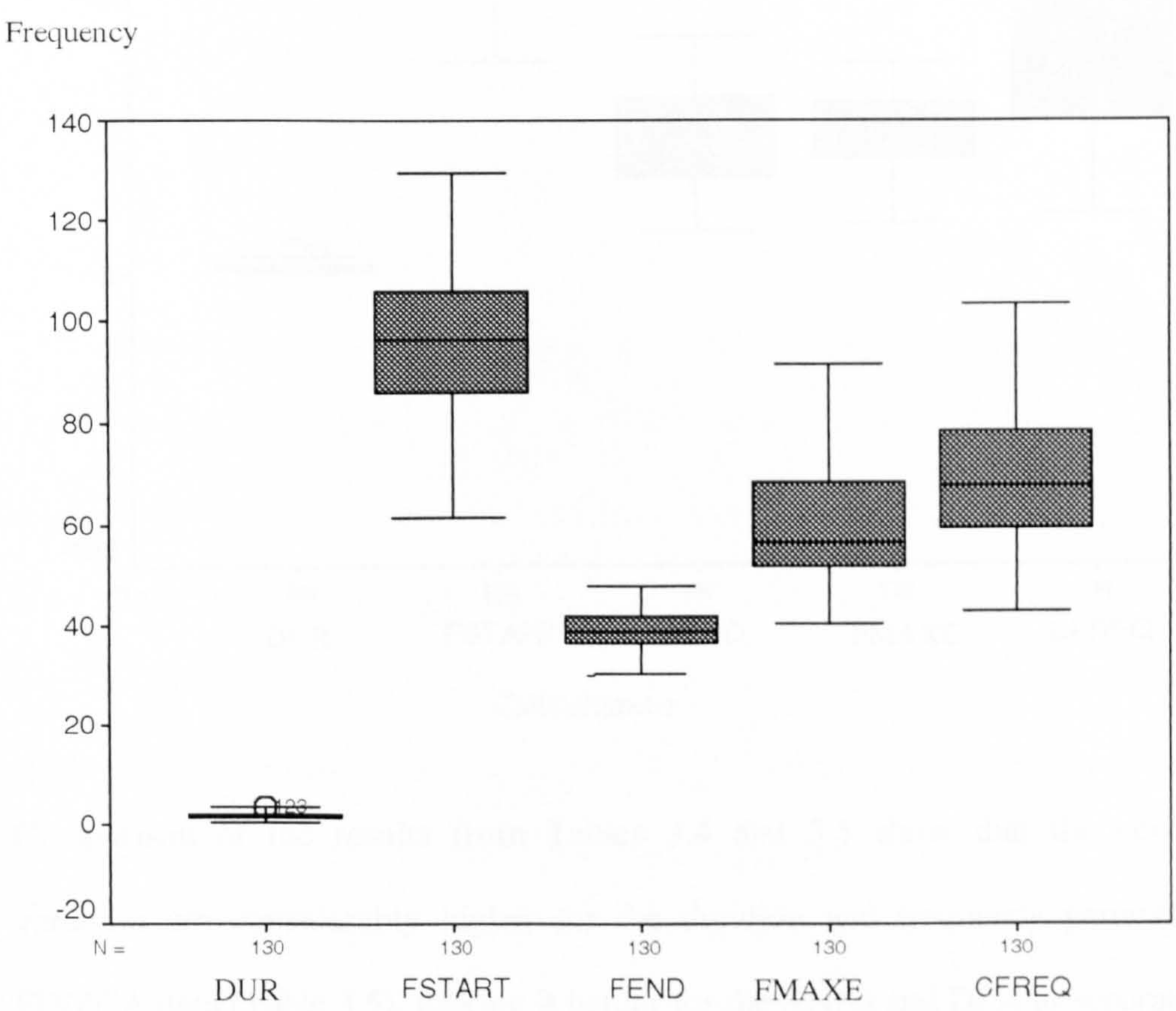
In order to understand why both ANNs and DFA were poor at classifying the FD/ZCA data, the variability in this data set was explored. Descriptive statistics were generated and coefficients of variation were calculated for each data set.

TE/FFT data

Table 3.4 Descriptive statistics and coefficients of variation for TE/FFT call parameters.

Parameter		***N***	**Mean**	**SD**	**CV %**
Duration	(ms)	130	1.84	0.60	32.6
Start frequency	(kHz)	130	95.98	15.72	16.4
End frequency	(kHz)	130	39.39	5.08	12.9
Frequency of maximum energy	(kHz)	130	59.79	10.12	16.9
Central frequency	(kHz)	130	69.33	12.26	17.7

Fig. 3.7 Box and Whisker plots of the TE/FFT *Myotis* data.

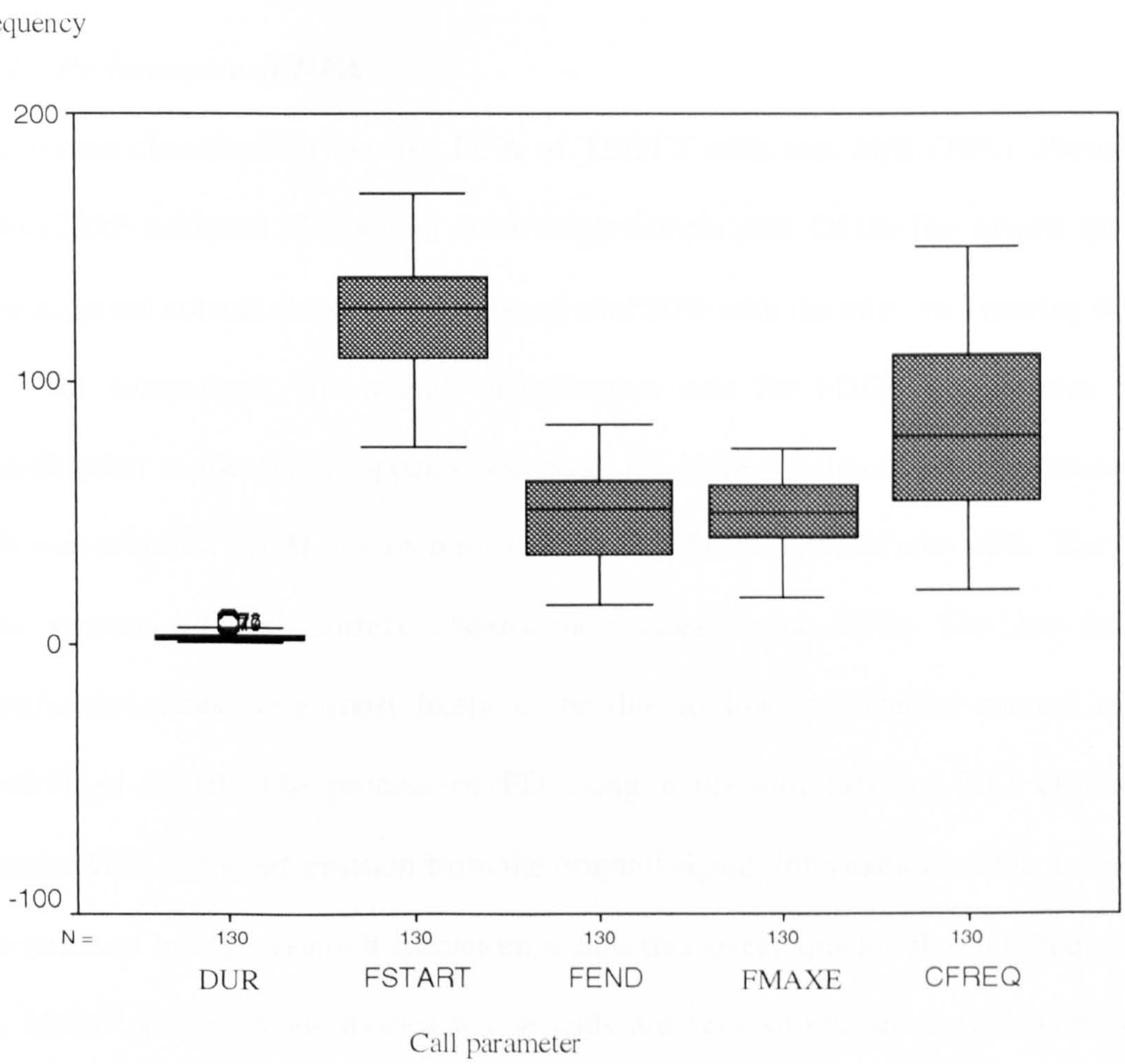


FD/ZCA data

Table 3.5 Descriptive statistics and coefficients of variation for the FD/ZCA data.

Parameter		*N*	Mean	SD	CV %
Duration	(ms)	130	3.65	1.70	46.6
Start frequency	(kHz)	130	123.97	21.88	17.6
End frequency	(kHz)	130	49.50	16.76	33.9
Frequency of maximum energy	(kHz)	130	53.89	24.29	45.07
Central frequency	(kHz)	130	81.01	31.84	39.30

Fig. 3.8 Box and whisker plots of the FD/ZCA *Myotis* data.



Comparison of the results from Tables 3.4 and 3.5 show that the coefficients of variation are considerably higher for the duration and frequency parameters of the FD/ZCA data (Table 3.5), making it harder for the ANNs and DFA to separate them.

The box and whisker plots highlight this point. Although there is some overlap in the 'whiskers' there is no overlap of the interquartile range of duration, end frequency and start frequency parameters for the TE/FFT data (Fig. 3.7). In contrast there is considerable overlap between the interquartile ranges of end frequency and frequency of maximum energy, as well as some overlap with central frequency for the FD/ZCA data (Fig. 3.8).

3.4 Discussion

3.4.1. Performance of DFA

The overall classification rate for DFA of TE/FFT calls was high (74%). Parsons & Jones (2000) achieved 79% with a much larger sample size. Of the five *Myotis* species, three achieved correct classification rates of over 70% with the other two nearing 60%.

In comparison, the overall classification rate for FD/ZCA calls was 52%. Classification of the *Myotis* species was poor; the highest correct classification rate of 60% was achieved for *M. mystacinus*, followed by *M. bechsteinii* with 48%. The other three species all had correct classification rates below 30%. The low correct classification rates were most likely to be due to low information content of the transformed signal. The process of FD using a division ratio of 10:1 effectively removed 90% of the information from the original signal. Information within a signal is also removed by this system if species emit calls that sweep quickly through frequencies e.g. *Myotis* species. With species whose calls are very similar in design, such as the *Myotis* species (Krusic & Neefus 1999), if large amounts of information are removed from the original signal, systems like Anabat (a commonly used frequency division system) may be removing the very information needed to separate these species (Parsons *et al.* 2000), a factor which may contribute to the poor species identification

results for this genus. The frequency response of microphones in commercially available FD detectors is not as flat as with TE systems, and the sensitivity of such microphones is less (Fenton 2001), and so classification rates from systems such as Anabat maybe even worse than those reported in this chapter.

Exploration of the *Myotis* data revealed high levels of intraspecific variation within the *Myotis* species for the FD/ZCA parameters, which led to overlap between species, making classification difficult.

3.4.2. Artificial neural networks – a comparison

ANN out-performed DFA in overall species classification rates for the TE/FFT data. The rate for classification to genus was also higher for ANN than the rate achieved for DFA, although both results were quite respectable, in the low 90% region. It should be noted that ANNs did better than DFA with effectively smaller data sets. For DFA *n*-1 is used to train the system. With ANNs only 50% of the data are used to train the network, the remaining 50% being used to test the network. This explains the poor identification rate for *M. brandtii* for the single ANN (0%) compared to the result from the DFA.

For the FD/ZCA data, DFA again produced lower classification rates compared to ANN, 52% and 70% respectively. The rates of classification to genus were also higher for ANN compared to DFA. The ability of ANNs to partition the work load and the benefits of this 'adaptability' was evident in the genus-specific hierarchical networks, which provided the best classification rates for species, especially for *Myotis* species with overall rates of 81% for TE/FFT compared to 52% for the FD/ZCA data set. The reason for the better results with the hierarchical genus-specific architecture is that the variability in the data set has been partitioned. The single large network has to make large-scale decisions e.g. genus, as well as fine-scale decisions (e.g. *M. brandtii*

vs *M. mystacinus*) decisions, which is a lot to ask of one network. By using the hierarchical approach, one network is specialised to make large-scale decisions whilst others make fine-scale decisions. As each network is more specialised, the decision making becomes more accurate. The poor classification of the *Myotis* species by both classification methods with the FD data could be explained by the higher variation found within the FD data. TE had lower within but higher between species variation making classification with this data set easier than with the FD data set.

3.5 Summary - the chosen system

Based on the results from Chapters 2 and 3, the possible use of FD calls for detailed call description and species identification for the bat study was rejected.

- Artificial neural networks consistently outperformed discriminant function analysis as a method of classification, and TE calls achieved higher rates of classification than FD calls due to their high information content.
- The classification rates of species which are difficult to separate due to similarities in call design, such as the *Myotis* species, were higher with ANN than with DFA, an important consideration when investigating species-specific habitat preferences.
- Although time expansion has been widely used during the past decade, direct sampling of ultrasound is only recently becoming possible in the field with the development of fast-sampling analogue digital cards that fit into laptop computers (Jones *et al.* 2000).
- For the farm study reported in this thesis, I was able to use direct sampling. This overcomes the main disadvantage of loosing recording time, a problem inherent in TE systems (Jones *et al.* 2000), whilst maintaining the same or better call

quality in terms of information content due to higher sampling rates. Direct sampling is a technologically more advanced and improved method compared to time expansion recording systems.

In the next Chapter, ANNs were used on directly sampled calls for the purpose of bat species identification to evaluate the impact of agricultural intensification on bat populations.

CHAPTER 4

BAT ACTIVITY AND SPECIES RICHNESS ON ORGANIC AND CONVENTIONAL FARMS: IMPACT OF AGRICULTURAL INTENSIFICATION

A paper based on this chapter has been published as: Bat activity and species richness on organic and conventional farms: impact of agricultural intensification. *Journal of Applied Ecology* **40**, 984-993.

4.1 Introduction

4.1.1. The status of Microchiroptera in the UK

Growing evidence suggests that many bat species are declining across Britain and Europe (Stebbings 1988; Harris *et al.* 1995; Mitchell-Jones 1995; Walsh & Harris 1996a, b; Hutson, Mickleburgh & Racey 2001). The status of bats has received increasing attention at an international level, reflecting the importance of their role in biodiversity and ecosystems (Hutson *et al.* 2001). Of the 16 species found in Britain, the International Union for the Conservation of Nature's Red List published in 2000 identifies *Rhinolophus hipposideros, Myotis bechsteinii* and *Barbastella barbastellus* as Vulnerable. The Bern and Bonn Conventions on biological diversity have focused on the plight of bats, resulting in an Agreement on the Conservation of Bats in Europe and the European Community (EC) Habitats and Species Directives (Annexes II & IV). All bat species found in Britain are protected by law in the European Union as well as by national legislation.

Six of the 16 species of bat in Britain (*Pipistrellus* species, since identified as two species - *Pipistrellus pipistrellus* and *Pipistrellus pygmaeus* (Jones & Barratt 1999),

Myotis bechsteinii, *Rhinolophus hipposideros*, *Rhinolophus ferrumequinum* and *Barbastella barbastellus*) have UK Biodiversity Action Plans (BAPs) assigned to them (Anonymous 1995). The BAPs identify habitat loss and agricultural intensification as reasons for the decline of all six species. However, there are few data to show the impact of agricultural intensification on bat activity. Despite the lack of data to support the assumptions that agricultural intensification is a factor in bat population declines, the Department for Environment, Food and Rural Affairs has emphasised the need to incorporate the requirements of bats into agri-environment schemes (http://www.defra.gov.uk).

Over 76% of the land in Britain is used for agriculture (Robinson & Sutherland 2002) and all of the bat species found in Britain forage in agricultural landscapes. Organic farming is a production system in which the use of synthetic fertilisers, pesticides, growth regulators and livestock feed additives are largely excluded (Lampkin 1998). The organic certifying bodies (The Soil Association Certification Limited, Bristol House, 40-56 Victoria Street, Bristol BS1 6BY; Organic Farmers and Growers Ltd., The Elim Centre, Lancaster Rd, Shrewsbury, Shropshire SY1 3LE) have rigid criteria that restrict any chemical input and provide mandatory rules for the management of livestock and crops. The organic standards prohibit the use of agrochemicals and include recommendations for the management of non-crop areas, such as woodland and riparian habitats.

In this chapter I evaluate the extent to which agricultural intensification is implicated in bat population declines by investigating the effects of intensification on bat activity, species richness and habitat use on matched pairs of organic and conventional farms. I tested the hypothesis that the increased use of agrochemicals, a major component of agricultural intensification, has no effect on bat activity.

4.2 Methods

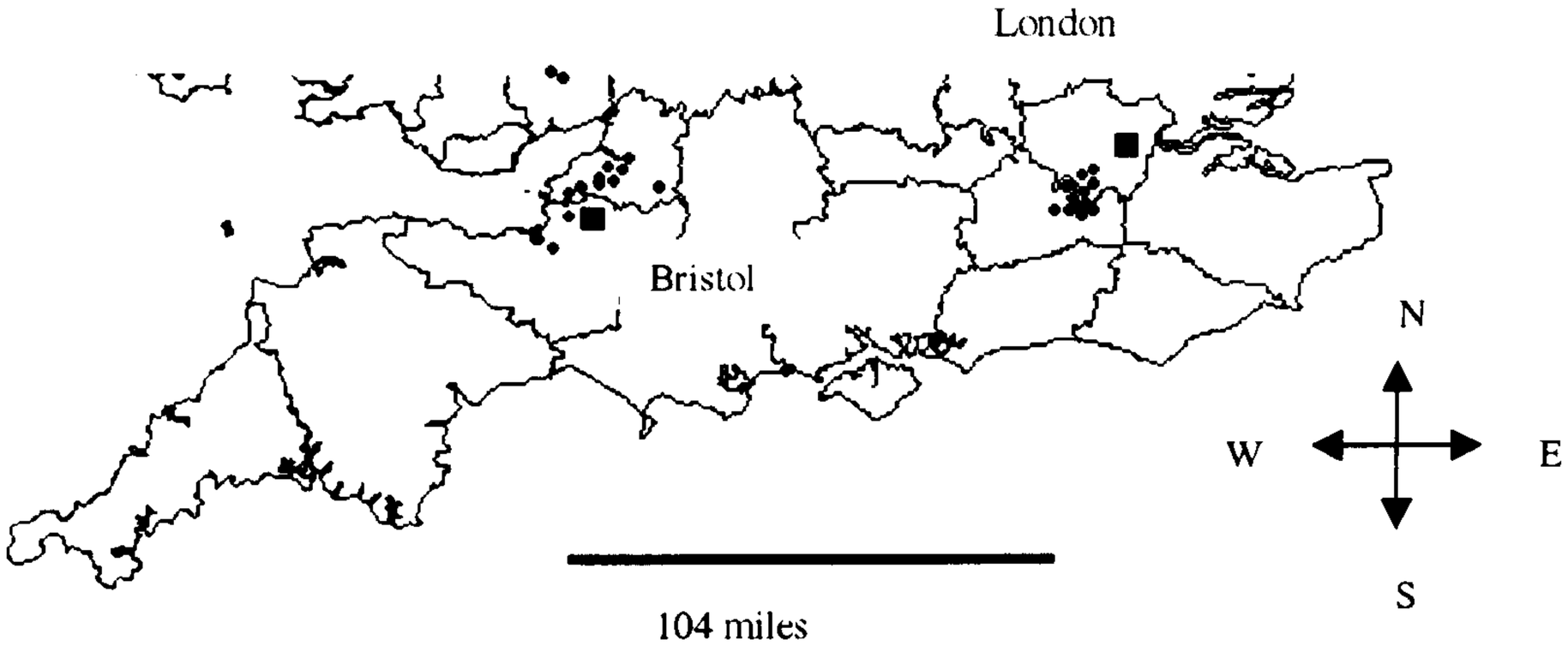
4.2.1. Study sites

The study was conducted from June to September in 2000 and 2002 on 24 pairs of farms in seven counties across southern England and Wales (Fig. 4.1). To reduce the likelihood that confounding variables would obscure any real differences due to farm type, sites were paired to standardise for various characteristics within a pair as much as possible with the exception of farm management. The organic farms used were those certified by the two official national certifying bodies. Certified farms are defined according to Soil Association and UK Register of Organic Food Standards after the completion of a 'conversion' period of two to three years of organic management. All the organic farms used had been established one or two years post conversion. As there is no national list of conventional farms, these were selected by asking the organic farmer about the nearest farm with a similar business that would be suitable for study. The business types of the 24 pairs of farms consisted of 54% mixed livestock and cattle, 41% mixed and 4% arable only. Further details on the farms can be found in appendix 8.1. Each organic farm was paired with a conventional farm no more than five km away to standardise for geographic variation. The sizes of the farms within a pair were similar, and each of the pair had to contain one or more of four previously selected habitats; these were pasture, arable land, water and woodland. Pasture, woodland and water habitats are known to be important habitats for bats (Walsh & Harris 1996a,b; Vaughan, Jones & Harris 1997). The water bodies selected for sampling were similar within a pair e.g. ponds or lakes. Rivers or streams running through farms were always the same within the pair.

4.2.2. Habitat surveys

Phase 1 habitat surveys (Anonymous 1990) were carried out on all farms. Geo-referenced tiles of all the study areas derived from digitised Ordnance Survey maps (http://digimap.edina.ac.uk) were put into a geographic information systems (GIS) application (Arcview version 3.2 and Arcview Spatial Analyst, Environmental Systems Research Institute, California, USA.), so that the habitat information could be merged with the base map tiles.

Fig. 4.1 Map of southern England and Wales showing the location of sites used to sample bats and insects. Each dot represents a pair of farms.



Using this GIS application habitat variables such as farm area, habitat area and length of hedgerow were calculated. If any of the selected habitats were present in both farms within a pair, they were sampled. The order of habitats surveyed within a pair was kept the same, but visits were randomised between pairs. On the night of sampling, habitat and environmental variables were measured at randomly selected sampling points within each habitat. These included temperature, wind speed, barometric pressure (Windwatch, Silva Alba, London, UK) and hedgerow height (1m ruler, accuracy ±1cm). Means of these measurements were calculated for each habitat for further analysis.

4.2.3. Sampling protocol

To avoid temporal differences in bat activity, the two farms of each pair were sampled within the period June to September on consecutive nights. This was not likely to introduce errors since variation in bat activity is thought to be greater within a night than between nights (Hayes 2000). Because sampling took place on consecutive nights, variations in weather had to be controlled for between nights and thus a strict sampling protocol was followed to standardise between pair comparisons. The temperature measured at dusk had to be within 4 °C of the previous night for sampling to take place on the second night. Insects become less active below 10 °C (Rydell, Entwistle & Racey 1996), and prolonged heavy rain would have damaged the sensitive equipment used to detect the bats, so sampling was abandoned if the temperature dropped below 10 °C or if heavy rain set in. If sampling was abandoned half way through the pair, the second farm was sampled on the next night following the same sampling protocol. If this second night was also unsuitable according to the protocol, then the pair of sites was re-sampled. This meant there was a gap of no more than one night between sampling farms in a pair.

4.2.4. Bat activity recording

Within each habitat, three points were chosen randomly for the acoustic survey of bat activity, points being more than 15 m apart. Sample points were marked 'blindly' on the habitat map and were often in different fields of the same habitat and in close proximity to a hedgerow, unless the habitat sampled was water or woodland in which case points were in close proximity to the habitat edge. Since individual bats cannot be counted with an acoustic method, bat activity was quantified by counting the number of bat passes (Fenton 1970) per 10 minutes at each point. This method was used to estimate intensity of use at survey points rather than abundance, although the two are almost

certainly correlated. Data from the three points were pooled for each habitat for analysis. Foraging activity was quantified by counting the number of feeding buzzes recorded (Griffin, Webster & Michael 1960). Bat sampling commenced one hour after sunset to avoid peak emergence times for different bat species and ended, on average, 1.5 hrs later. The length of sampling time varied between pairs depending on the number of habitat types present. The timing of sampling ensured that it coincided with the peak foraging activity for aerial foraging bats, and ended before insect abundance dropped (Racey & Swift 1985).

Bat activity was recorded sequentially during a sampling period of 10 minutes at each point, digitising ultrasound using a laptop computer (Toshiba, Satellite Pro, 4080XCDT, Toshiba of Europe, London, UK) with a PCMCIA III card (DAQCard AI-16E-4, National Instruments, Austin, Texas, USA; sampling frequency=500 kHz) connected to an S25 bat detector (Ultra Sound Advice, London). The detector was housed on a tripod one metre above the ground angled up at 45°. If the habitat was bordered by a hedgerow, the detector was also angled approximately 20° from this feature towards the field. Recording was triggered manually for five seconds whenever a bat call was heard on frequency division, and the bat passes sampled using BatSound software (BatSound v1.0, Pettersson Elektronik AB, Uppsala, Sweden). The recordings from the direct sampling method were used for quantification of activity and species identification.

A second method of recording bat activity was used simultaneously to obtain real-time recordings of feeding buzzes. A Pettersson D980 detector (Pettersson Elektronic AB, Uppsala, Sweden) or S25 detector was linked to a professional Walkman cassette recorder (Sony WM D6C, Sony, Tokyo) or a digital audio tape (DAT, TCD-D8, Sony, Tokyo) and the frequency-divided output was recorded

continuously for the duration of the 10 min sampling period. The same combination of equipment was used within each pair so that changes did not affect the analysis.

4.2.5. Bat species identification and statistical methods

The first call with a good signal to noise ratio was selected from each pass and entered into an artificial neural network program (ANN) (Parsons & Jones 2000). ANNs are relatively new techniques that have been applied to the identification of individuals and species (Burnett & Masters 1999; Parsons & Jones 2000). The ANN used for this study had already been developed at the University of Bristol by Stuart Parsons, where it was 'trained' using data sets of calls produced by known species, and programmed to classify calls down to genus and then species level with a reported degree of confidence associated with each identification (Parsons & Jones 2000). ANNs rarely give absolute confidence on species identification; the overall level of confidence used here was 85%, and if the confidence fell below this value the result was considered unreliable and not used in the species-specific analysis.

The differences between farm types were analysed using the paired *t*-test, if the differences were normally distributed. Data were log ($\log_{10}$ (X+1)) transformed if necessary to achieve normality in the differences (Zar 1999). Analyses were carried out using Minitab version 13 (Ryan & Joiner 1994). Organic data minus the conventional data were used to generate the differences; the direction was the same in all of the paired tests.

4.3 Results

1747 passes were recorded in 47 hours (Table 4.1); 89% were identified to species using the ANN. The 9% that could only be identified to genus consisted of *Pipistrellus*,

Myotis species and *Nyctalus* species which made up 44%, 48% and 8% of this value respectively. The remaining 2% of the calls could not be identified to genus or species.

No statistical difference was found between organic and conventional farms for mean temperature, mean wind-speed, total number of habitats (i.e. including those not sampled), farm area and areas of habitats sampled, confirming that the pairs used were comparable with respect to these characteristics. Hedgerow height was found to be significantly greater on organic farms compared with conventional farms (Table 4.2).

Table 4.1 Total sampling time in each habitat type and the corresponding total number of bat passes for farm type.

Habitat	**Total sampling time (hrs)**	**Total bat passes recorded**	
		Organic	**Conventional**
Pasture	21	335	246
Arable	8	74	58
Woodland	10	267	176
Water	8	447	144
Totals	**47**	**1123**	**624**

Table 4.2 Statistical comparison of habitat and environmental variables between organic and conventional farms. Not all farm pairs contained all habitat types hence sample size differs. Mean ±sd (minimum-maximum). *P* values derived from paired *t* tests.

Variable	**Farm type**		**d.f.**	***t***	***P***
	Organic	**Conventional**			
Wind speed (m/s)	0.2±0.3 (0-1.1)	0.4±0.5 (0-2.5)	23	-1.87	0.074
Temperature (°C)	14.7±2.2 (11-18)	13.7±2.3 (10-18)	23	1.98	0.059
Hedge height (m)	2.5±0.5 (1.4-3.6)	1.9±0.6 (1.2-3.4)	22	5.77	<0.001
Hedge length (km)	4.4±1.9 (1.4-8.9)	3.6±2.2 (1.3-10.9)	23	1.87	0.074
Farm area (ha)	47.2±29.0 (11.8-133.8)	55.3±28.0 (18.5-117.2)	23	-1.59	0.124
Pasture area (ha)	32.8±15.9 (9.0-75.2)	28.8±12.8 (13.0-53.4)	20	0.79	0.437
Arable area (ha)	33.0±17.1 (5.3-62.8)	35.8±18.5 (9.3-72.7)	7	-0.25	0.809
Woodland area (ha)	4.6±4.9 (0.3-14.9)	6.9±9.1 (0.5-29.5)	9	-0.44	0.672
Water area (ha)	1.6±1.6 (0.005-5)	0.7±1.1 (0.05-3.5)	7	-1.20	0.351
Total no. of habitats	3.6±1.2 (1.0-6.0)	3.4±0.9(2-6)	23	0.92	0.366

4.3.1. Bat activity

Total bat activity (all species) was significantly higher (by 61%) over organic farms than over conventional farms (t=2.38, d.f.=23, P=0.026; Fig. 4.2). When habitat types were analysed separately, significantly higher numbers of passes were found over water habitats only (Table 4.3). It should be noted that the directionality of the t values are consistent in all non-significant results (Table 4.3). Foraging activity, derived from feeding buzz counts from the real-time recordings, was significantly higher (by 84%) on organic farms (t=3.15, d.f.=23, P = 0.004; Fig. 4.3). The numbers of feeding buzzes per pass (buzz ratio) was also significantly higher on organic farms, indicating a higher foraging effort on this farm type (t=2.61, d.f.=23, P=0.016) (Fig. 4.4). There was a significant correlation between the number of feeding buzzes and hedgerow height (Spearman's coefficient correlation, r_s=0.354, n=48, P=0.016). No significant differences in foraging activity within individual habitats were found between farm types (Table 4.3).

Fig 4.2 Differences in total numbers of bat passes per pair of organic and conventional farms. For Figs 4.2-4.4 bars in black indicate more passes, feeding buzzes or higher buzz ratio over organic farms; white bars indicate more passes, feeding buzzes or higher buzz ratio over conventional farms.

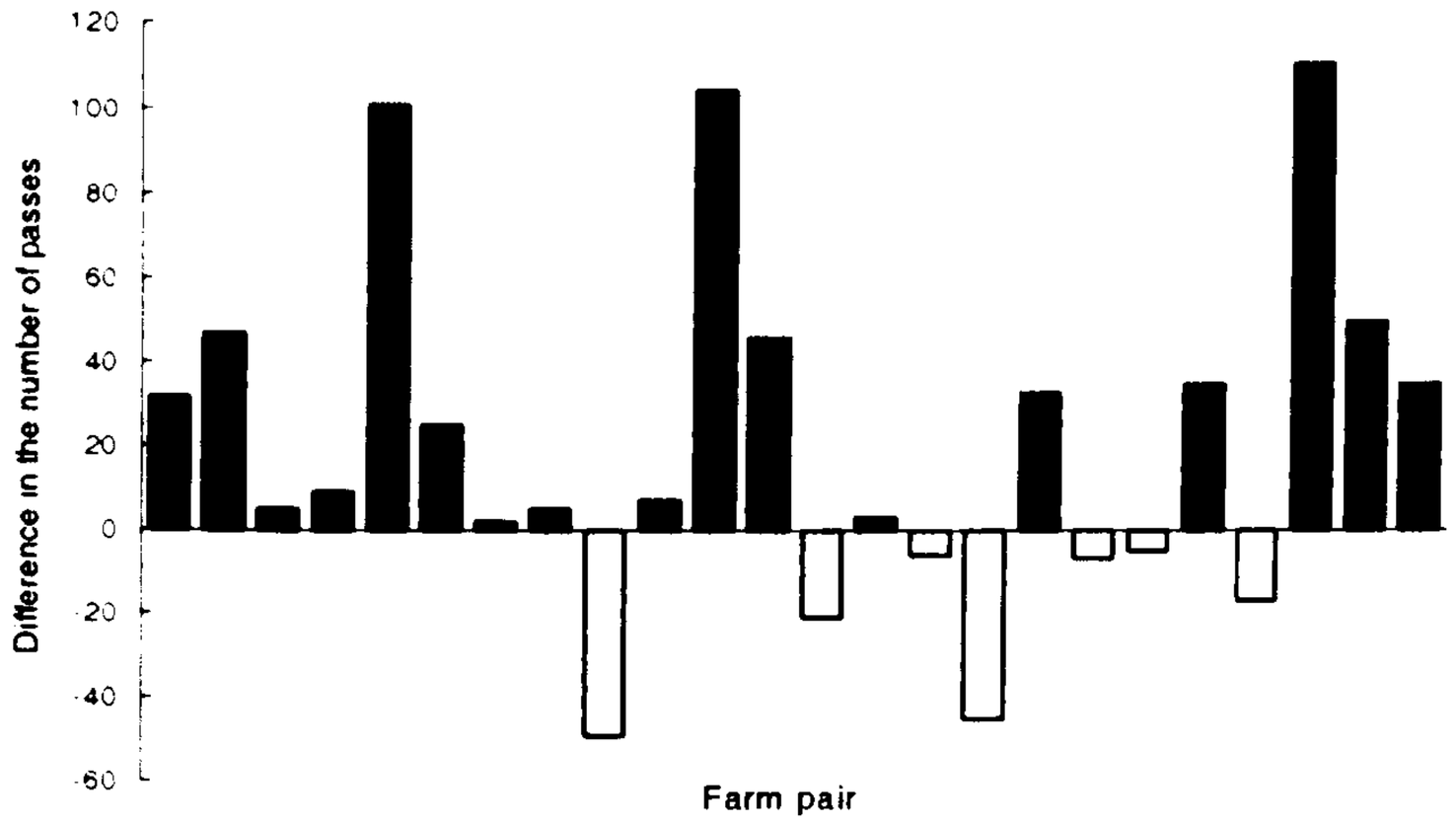


Fig. 4.3 Differences in total numbers of feeding buzzes per pair of farms.

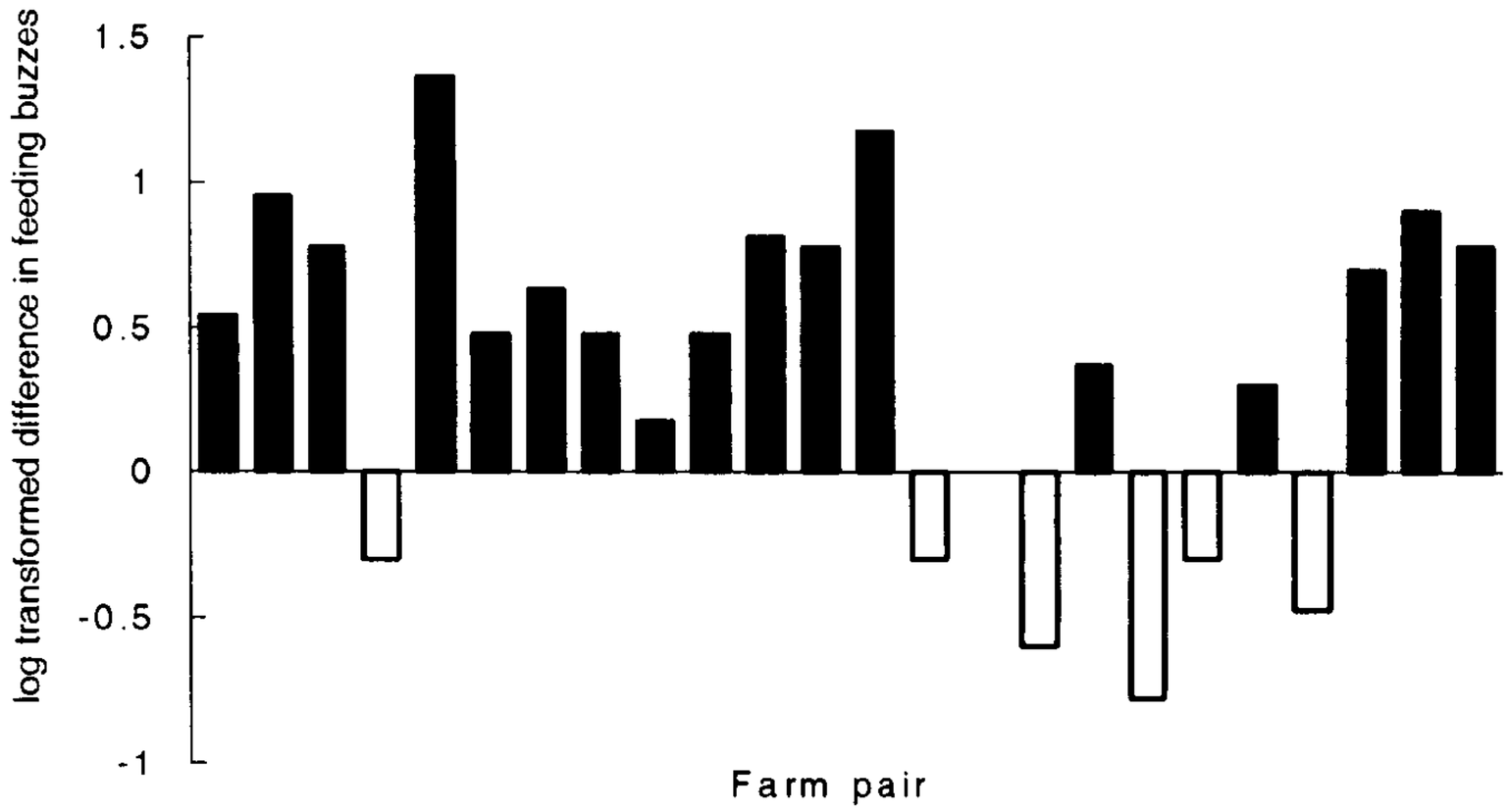


Fig. 4.4 The differences in buzz ratio per pair of farms.

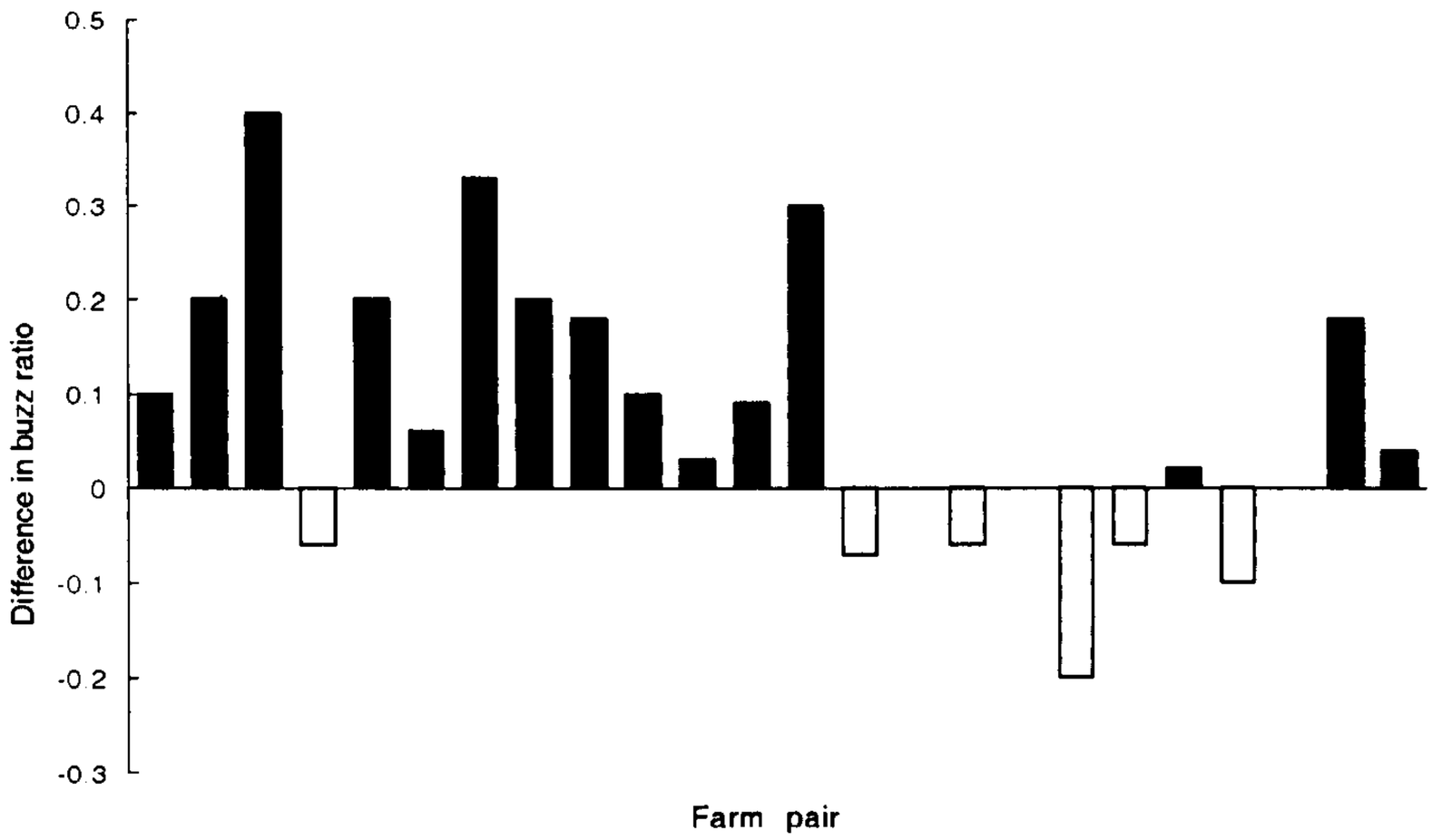


Table 4.3 Statistical significance of differences in bat activity between organic and conventional farms. Buzz ratio = number of feeding buzzes per bat pass.

	N	*T*	*P*
Passes-pasture	21	0.85	0.406
Passes-arable	8	0.68	0.516
Passes-woodland	10	0.48	0.642
Passes-water	8	2.51	0.040
Feeding buzzes-pasture	21	1.76	0.094
Feeding buzzes-arable	8	1.44	0.192
Feeding buzzes-woodland	10	1.67	0.139
Feeding buzzes-water	8	1.61	0.141

Buzz ratio-pasture	21	1.69	0.106
Buzz ratio-arable	8	0.54	0.603
Buzz ratio-wood	10	1.15	0.281
Buzz ratio-water	8	0.12	0.909

4.3.2. Bat species composition

Species richness was not statistically significantly different between farm type. Fourteen of the 16 British bat species were detected on organic farms compared to 11 on conventional farms (Figs 4.5 a and b). In both farm types *Pipistrellus pipistrellus* had the highest activity levels with *Pipistrellus pygmaeus* being the second most frequently detected species. Both species made up over 70% of all passes for both farm types. Species composition differed between organic and conventional farms. *Rhinolophus* species were only detected on organic farms, with 11 *Rhinolophus hipposideros* passes and one *Rhinolophus ferrumequinum* pass. Significantly higher *Myotis* activity was recorded on organic farms than on conventional farms (t=2.62, d.f.=23, P=0.015). When all the species data were considered in all habitats, the activity of both *M. daubentonii* (t=2.09, d.f.=23, P= 0.048) and *M. brandtii* (t=2.27, d.f.=23, P=0.033) were significantly higher on organic farms than on conventional farms. Over 50% of the passes by both of these species were recorded over water habitats.

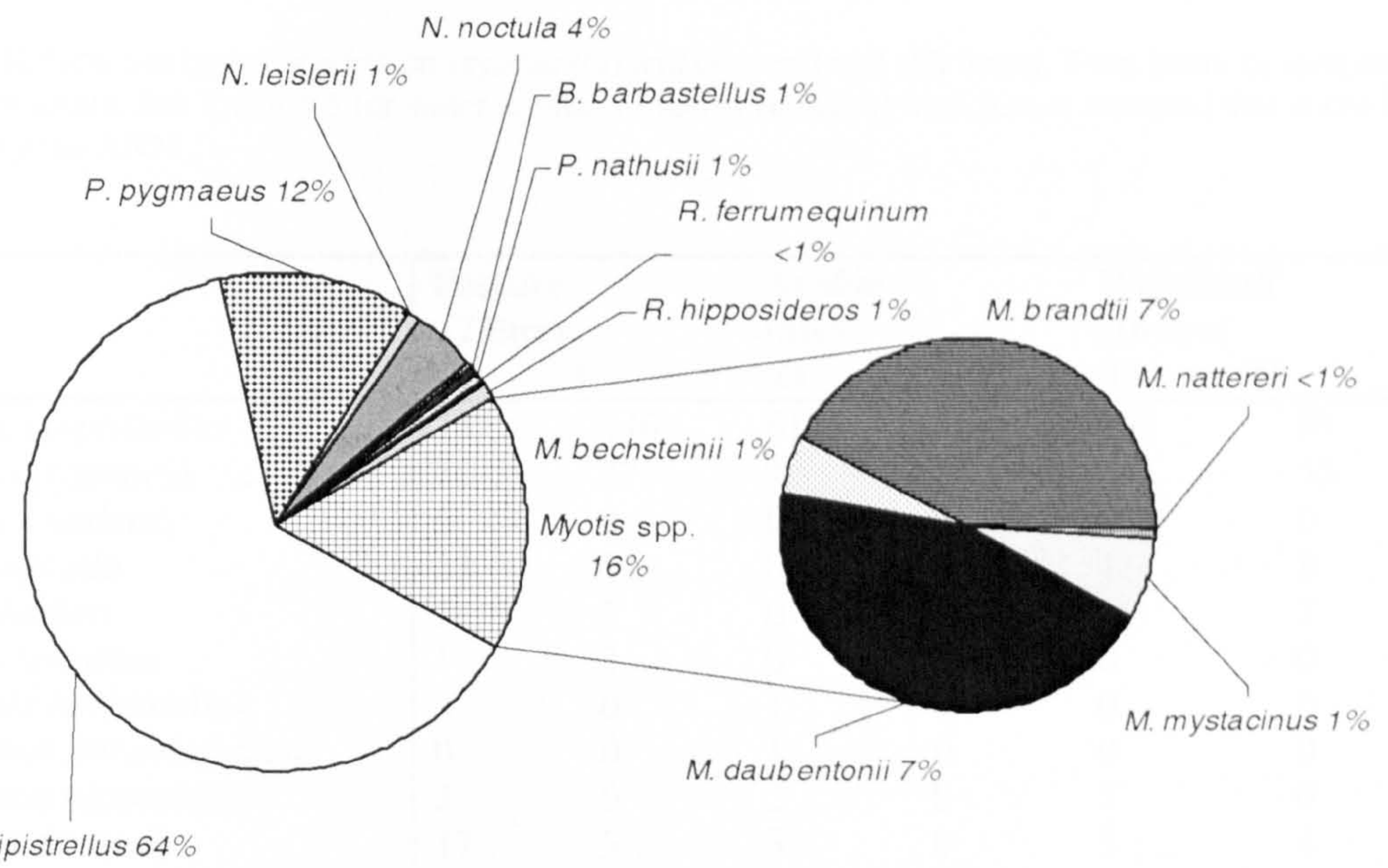
4.3.3. Habitat use

The activity of individual species recorded on organic and conventional farms within different habitats is summarised in Table 4.4 *Rhinolophus hipposideros* was only recorded on organic farms in pasture, arable and woodland habitats, with the majority being in woodland habitats. The only recorded pass of *Rhinolophus ferrumequinum* was over organic arable habitat on one farm. *Nyctalus noctula* was recorded in all habitats on both farm types, with the exception of organic arable, but was predominantly found

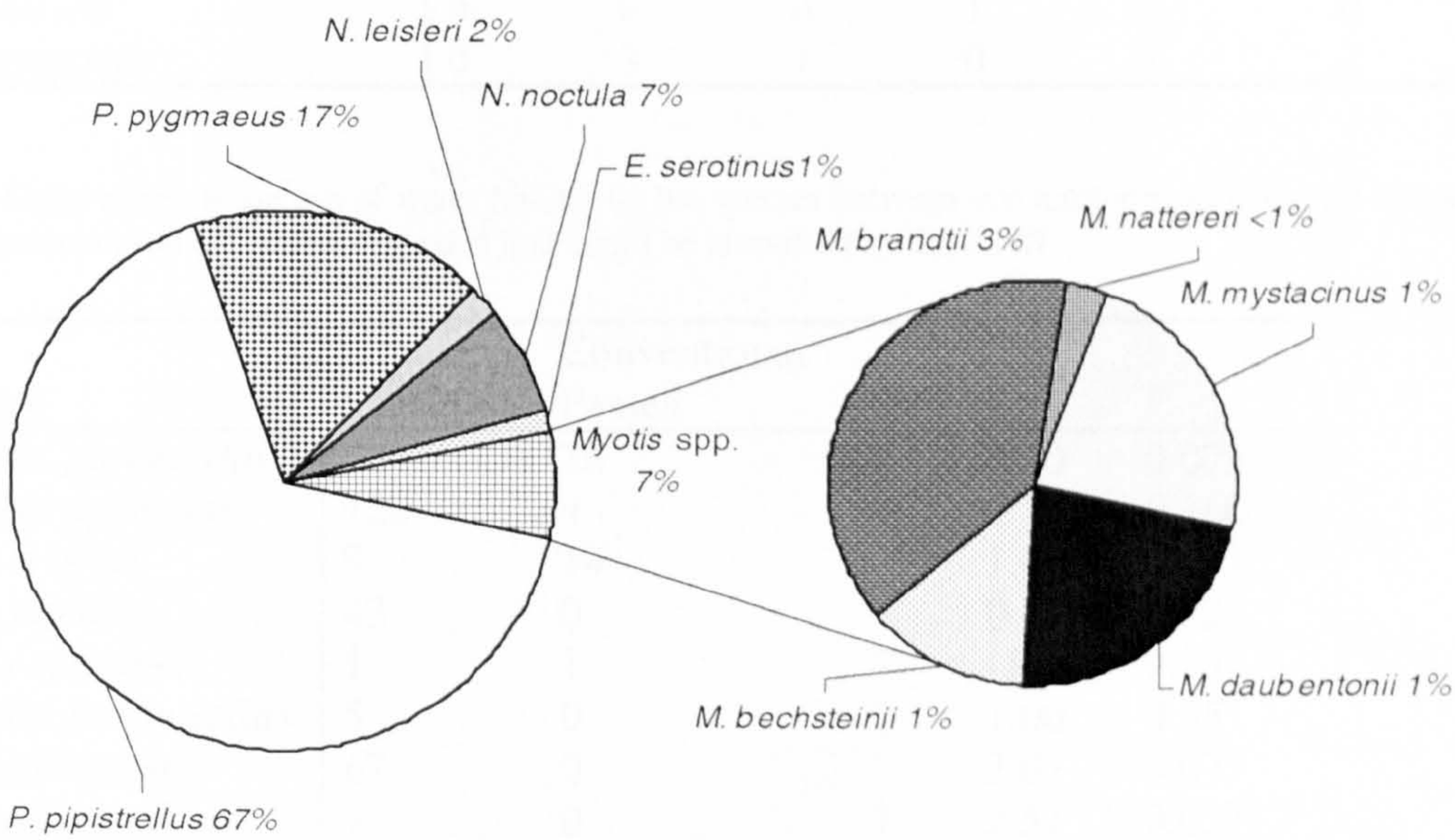
over pastural and water habitats. Activity of *Myotis* species over water habitats was significantly higher (t=3.47, d.f.=7, P=0.01) on organic farms than in the same habitat on conventional farms

Fig. 4.5 a & b Species composition for bats recorded on organic farms (a) and conventional farms (b) classified by the ANN (a, n=976; b, n=574).

a)



b)



All species of *Myotis* were recorded over water bodies on organic farms, although the numbers of passes of *M. bechsteinii* and *M. brandtii* were significantly higher on water bodies on organic farms than on water bodies on conventional farms (Table 4.5). The only *Myotis* species recorded over conventional water habitats was *M. brandtii* (n=2).

Table 4.4 Habitat use by bat species on organic (O) and conventional (C) farms. Total hours of sampling shown in brackets. See Table 4.5 for water habitats. Figures represent total passes recorded that could be classified by the ANN.

Species	**Pasture (21hrs)**		**Arable (8hrs)**		**Woodland (10 hrs)**	
	O	**C**	**O**	**C**	**O**	**C**
Pipistrellus pipistrellus	207	146	61	48	152	89
Pipstrellus pygmaeus	61	77	7	7	54	55
Pipistrellus nathusii	6	0	0	0	0	0
Nyctalus noctula	23	14	0	2	1	8
Nyctalus leisleri	5	7	0	2	4	2
Eptesicus serotinus	1	3	0	2	0	0
Barbastella barbastellus	2	0	1	0	0	0
Rhinolophus ferrumequinum	0	0	1	0	0	0
Rhinolophus hipposideros	2	0	2	0	7	0
Myotis daubentonii	17	5	3	0	3	4
Myotis bechsteinii	0	3	0	0	0	1
Myotis brandtii	24	8	3	2	2	2
Myotis nattereri	0	0	0	1	0	0
Myotis mystacinus	0	3	1	0	3	2

Table 4.5 Differences in the use of water habitat by bat species between conventional and organic farms. Figures represent total bat passes recorded that could be classified by the ANN.

Bat species	Organic Passes	Conventional Passes	d.f	T	P
Pipistrellus pipistrellus	179	78	7	2.10	0.074
Pipistrellus pygmaeus	122	4	7	1.57	0.160
Nyctalus leisleri	9	14	7	1.00	0.351
Nyctalus noctula	43	0	7	0.37	0.725
Eptesicus serotinus	1	1	7	-1.00	0.351
Barbastella barbastellus	5	0	7	1.00	0.351
Myotis daubentonii	67	0	7	2.07	0.077
Myotis bechsteinii	7	0	7	2.57	0.037
Myotis brandtii	66	2	7	2.54	0.039
Myotis mystacinus	12	2	7	1.08	0.316

4.4 Discussion

4.4.1. Paired design

Two important factors to consider when dealing with a paired design are sample size and sampling protocol. The number of paired sites needed and how often they are sampled depends not only on the sample size required for statistical power, but also on the nature of the organism being sampled.

Bats are highly mobile animals covering several kilometres in a single night. They follow flight paths along landscape features, such as hedgerows and edge habitats (Racey & Swift 1985; Walsh & Harris 1996a, b; Verboom & Huitema 1997; Grindal & Brigham 1998; Verboom & Spoelstra 1999). A large number of farm pairs were studied, sampling each farm once. The experimental design minimised any bias due to sampling in favoured flight paths by including a large number of farms (24 pairs) and by having multiple sample points chosen randomly within habitats. Also, the farms studied covered a large geographical area to represent regional differences in farm management.

This type of paired experimental design has been widely used in comparing aspects of organic and conventional farms (Feber *et al.* 1997; Chamberlain *et al.* 1999; Letourneau & Goldstein 2001). Chamberlain *et al.* (1999) found that when geographical location and observer differences were not standardised between farm pairs, such differences accounted for as much as 60-80% of the variation in the species data. In this study, geographic location was standardised since farms within a pair were no more than 5 km apart. The 'mixed' nature of organic cropping systems and their habitat management guidelines means that organic farms are more diverse in terms of habitat types. They are often smaller and have a greater species richness of livestock and non-crop flora compared with conventional farms (Mansvelt, Stobbelaar & Hendriks 1998). Benton *et al.* (2003) recently argued that a reduction in habitat heterogeneity due to

agricultural intensification is a major factor in farmland biodiversity declines. However, in this study no difference in farm area, total number of habitats, or area of habitats sampled between the two farm types were found. These results reflect the effectiveness of the paired experimental design used for this study.

Nightly bat activity is variable, and many studies have shown that the highest activity peaks occur early in the night (Park, Jones & Ransome 1999; O'Donnell 2000; Kuenzi & Morrison 2003). The present study mainly considered those aerial hawking species producing detectable echolocation calls. Their prey, aerial insects, are known to peak in abundance early in the night, thereafter decreasing rapidly and reaching lowest numbers in the middle of the night (Racey & Swift 1985). Although there are biases associated with sampling only within a specific time period each night, and some authors recommend monitoring throughout the night (Kuenzi & Morrison 2003), the timing of the sampling coincided with the highest activity, especially for the aerial hawking insectivores that were the main focus of the study. Sampling also started after the main emergence times, thereby reducing any bat species bias or proximity to roosts, which should be independent of farm type.

Until recently it has been difficult to quantify accurately the differences between bat species from their echolocation calls (Walsh & Harris 1996a, b), and previous methods could usually only identify bats to genus. The use of the most recent advances in echolocation recording technology resulted in 89% of the calls being identified to species. This was important for discriminating species differences in habitat use, a crucial aspect of this study. However, there is an inherent problem with all acoustic methods in that not all species are detected equally. Those species with very low amplitude calls, such as the *Plecotus* species, may not be detected adequately (Vaughan *et al.* 1997). This may explain why this genus was under represented in the data.

However, the 9% of calls that could only be identified to genus by the ANN were not biased towards any one genus, with over 40% from each of *Myotis* and *Pipistrellus* species.

4.4.2. Impact of agricultural intensification on bat species

The most important and large-scale cause of habitat fragmentation is the expansion and intensification of land-use (Burgess & Sharpe 1981). Conventional farming systems fragment the wider landscape into a matrix or mosaic. Within this mosaic, organic farms have more characteristics in common with semi-natural habitats compared with the surrounding intensively farmed landscape, and may therefore be more attractive to a number of species. Fragmenting a large area of habitat into a mosaic may be beneficial to certain bat species by increasing edge habitat, although it will be detrimental to others by decreasing linear features connecting foraging areas (Russ & Montgomery 2002). This will isolate populations and remove access to suitable foraging sites.

Bright (1993) used life history traits of British mammals to arrange species according to their potential response to habitat fragmentation. He proposed that generalist species use a wide range of habitats and are therefore less likely to be dramatically affected by habitat fragmentation, compared with specialist species, which would be more vulnerable to the adverse effects of habitat fragmentation. Bright (1993) found that specialist species of bat, with those most likely to be affected by habitat fragmentation listed first, included *Myotis myotis*, both *Rhinolophus* species, *M. daubentonii*, *M. nattereri* and *M. mystacinus*. The generalists were identified as *Nyctalus noctula*, *Eptesicus serotinus*, *Pipistrellus pipistrellus* and *Plecotus auritus*. I only recorded *Rhinolophus* activity on organic farms, and *Myotis* activity was significantly higher on organic farms compared with conventional farms. In Britain the

numbers of *Rhinolophus hipposideros* seem to be increasing, although there is particular concern about this species in Europe, where it is threatened with extinction in East and West Germany and is in severe decline over the rest of Europe (Hutson *et al.* 2001). The more generalist species *N. noctula* and *P. pipistrellus* were found on both farm types. Thus, the results presented support Bright's (1993) predictions and suggest that the species adversely affected by habitat fragmentation are also adversely affected by agricultural intensification.

4.4.3. Changes in bat populations and agricultural intensification

Bat activity overall was 61% higher on organic farms, and foraging activity was 84% higher on organic farms, suggesting that bats preferred the organic farms over conventional farms for both foraging and general movements. The importance of linear features within a landscape has been well documented for bats (Verboom & Spoelstra 1999). Whether the features are walls, woodland edges or hedgerows, bats use them as flight paths and foraging sites if the feature provides enough shelter for insects to aggregate. With the main focus on aerial hawking bats, hedgerow height was thought to be more important in terms of shelterbelts for insects and bats than hedgerow width. Insect densities are generally higher nearer vertical landscape elements (Lewis & Stephenson 1966; Lewis & Dibley 1970; Verboom & Spoelstra 1999). A significant correlation was found between the number of feeding buzzes and hedgerow height, supporting the hypothesis that the significantly higher hedgerow height recorded on organic farms contributed to the higher bat foraging activity on organic farms.

Habitat quality may well be important in explaining the differences seen between farm types. Riparian habitats are critical habitats for many bat species (Brigham & Fenton 1991; Rydell *et al.* 1994; Racey 1998; Grindal, Morissette &

Brigham 1999). In conjunction with higher total bat activity over water habitats on organic farms, the activity of *Myotis* species was significantly higher. Water habitats are important foraging areas for *Myotis* species. Water quality is affected by agrochemicals (Racey *et al.* 1998) and a direct link between agricultural intensification and water quality has been reported in Canada (Berka, Schreier & Hall 2001). Eutrophication of water habitats from sewage outlets can increase abundance in some insects and may benefit some species of bat (Vaughan, Jones & Harris 1996), whereas some agricultural pollutants may have a detrimental effect on the insects found in and around water habitats, thus affecting food availability for bats. Increasing the nutrient content of water bodies may result in seasonal changes in invertebrates with consequences for organisms higher in the food web (Mason 2002). Agrochemicals applied to fields, particularly nutrients (nitrogen and phosphorous) and pesticides, are a major form of aquatic pollution (Angier *et al.* 2002). Excess phosphorous loading primarily affects aquatic life, whereas excess nitrogen may affect both aquatic life and human health (Hapeman 2002). Organic pollution from fertilisers or slurry results in a reduction in the oxygen content of the water, adversely affecting some sensitive invertebrates such as trichopteran larvae and Plecoptera, although the actual impact depends on the severity of oxygen depletion (Mason 2002). Sites of organic pollution may also show increased activity of other insects such as chironomids, as the oxygen levels increase (Mason 1996). Thus, the use of agrochemicals may explain the differences in bat activity over water habitats between farm types, and implies that localised changes in water quality may account for differences in bat activity.

This part of the study was designed to determine whether agricultural intensification had been a factor in British bat population declines. The answer is a tentative yes and the experimental hypothesis can be rejected. The results presented

suggest that, compared with similar areas on conventional farms, the habitats found on organic farms are possibly higher in quality in terms of habitat structure and condition (due to the lack of agrochemicals), than the same habitats on conventional farms, making them favourable foraging sites for bats.

4.5 Summary

- Bat activity was 61% higher on organic farms than on conventional farms
- Foraging activity was 84% higher on organic farms
- Particular bat species benefit from organic farming, including many with UK BAPs
- Differences in bat activity maybe due to factors such as taller hedgerows on organic farms and better quality water habitats due to the lack of agrochemicals in the system

In the next Chapter I investigate functional reasons as to why bats are less common on conventional farms by studying the effect of agricultural intensification on their prey.

CHAPTER 5

NOCTURNAL INSECT ABUNDANCE AND SPECIES RICHNESS ON ORGANIC AND CONVENTIONAL FARMS: IMPLICATIONS FOR BAT FORAGING

Work based on this chapter is in press as: Abundance and species richness of nocturnal insects on organic and conventional farms: implications of agricultural intensification for bat foraging, *Conservation Biology*. See appendix 3.

5.1 Introduction

Insects are the principal food for many animals, including all the British bats (Chiroptera). Intensive farming is multifaceted, and as well as utilising agrochemicals for insect removal, other features such as habitat modification also affect insect communities.

Many insect species have shown marked population declines over recent years, most of which have been attributed to agricultural intensification (Aebischer 1991; Feber *et al.* 1997; Benton *et al.* 2002). There have been declines in a number of carabid species in Europe (Luff & Woiwood 1995; Kromp 1999), and studies of long-term trends in invertebrate abundance in Britain showed that most insect groups have declined, notably Collembola, carabids and other predatory insects (Aebischer 1991; Sotherton & Self 2000).

Few data exist on the mechanisms by which agricultural intensification has an impact on bat populations; in particular, little is known about the effect of agricultural intensification on the predominantly nocturnal insect taxa most likely to be eaten by bats.

5.1.1. The insect prey of bats

All species of bat in Britain include Lepidoptera as a component of their diet (Vaughan 1997). Ninety percent of lepidopteran species are moths, the vast majority of which are nocturnal (Janzen 1988; Young 1997). Many species of bat feed predominantly on Lepidoptera (Vaughan 1997). Lepidoptera constitute one of the main dietary components for six species of British bat, three of which (*Rhinolophus ferrumequinum*, *Myotis bechsteinii* and *Barbastellus barbastella*) have UK BAPs. As well as featuring heavily in the diet of certain bat species, Lepidoptera play important roles as herbivores and pollinators (Janzen 1987; Barlow & Woiwood 1989). Their host-specificity means that they can also act as indicators of plant diversity and land management (Erhardt & Thomas 1991; Luff & Woiwood 1995).

Some Trichoptera are also eaten by all the bat species. *Myotis* spp., *Pipistrellus* spp. and *Nyctalus leisleri* are highly reliant on aquatic insects, predominantly dipteran flies (Swift & Racey 1983; Swift *et al.* 1985; Barlow 1997; Vaughan 1997). In the UK, Diptera constitute a major part of the diet of 13 species of bat, of which four have BAPs assigned to them. Scarabid and geotrupid beetles form a major component of the diet for some of the larger species of bat, notably *Rhinolophus ferrumequinum*, *Nyctalus noctula* and *Eptesicus serotinus* (Hutson *et al.* 2001). Within the agricultural context, bats are predators of a number of pest species, making them beneficial in terms of pest control (Murphy 1993; Long 1996).

5.1.2 Aims of this chapter

Many facets of the biology of bats, including low fecundity, longevity and high survivorship, indicate that they should maintain stable populations close to the carrying

capacity of the environment within predictable habitats. These biological traits also suggest that bat populations are limited by resources (Findley 1993).

Nocturnal and crepuscular aerial insects were sampled on the same 24 matched pairs of organic and conventional farms described in the previous Chapter. The aims were to examine the relationship between the abundance and species richness of insects and farm type, to assess the impact of agricultural intensification on specific insect families known to be important in the diet of bats (referred to as "key" insect families) and to investigate the relationship between bat activity and insect abundance. To address these aims I tested the hypothesis that nocturnal insect prey is equally abundant on organic and conventional farms.

5.2 Methods

5.2.1. Study sites and sampling protocol

Details of study sites, habitat surveys and sampling protocol are given in Chapter 4. The sizes of the farms within a pair were kept as similar as possible and insects were sampled within all the habitats used for bat sampling. As bats were being sampled simultaneously, temporal differences in bat activity were controlled for by sampling on consecutive nights following a strict sampling protocol. This protocol ensured that environmental variables were controlled for between nights.

5.2.2. Insect capture methods

As all insect capture methods are biased towards catching prey of a certain size, mass or flight behaviour (Muirhead-Thompson 1991; Sutherland 1998), a combination of portable Heath light traps (Alana Ecology), flight intercept traps and sweep netting (Marris House Nets) was used. The Heath trap used a blacklight bulb as the attractant, powered by a 12-volt motorcycle battery. Three hundred sweeps with a sweep net were

made in a figure of eight within each habitat type, 100 sweeps at three randomly chosen sampling points no less than 15 m apart. The flight intercept trap consisted of a fine black net (53x97 cm) stretched between two poles and placed at a 90° angle to the wind direction. Fast flying insects hit the netting and fell into a tray (35x54cm) containing a weak solution of detergent. One of each type of trap was placed near a hedgerow within each habitat sampled, each trap >15 m from the next. On most occasions the traps could be placed in different fields of the same habitat.

The timing of sampling was important as bat activity was also being sampled. In order to standardise sample collection, insect traps were activated in each of the selected habitats at dusk, and the catch was collected when the night's sampling had ended (typically after five hours including time moving between bat sampling points). The timing of insect sampling coincided with peak foraging activity of bats and ended before insect abundance dropped (Racey & Swift 1985). At the end of the bat sampling, the insect traps were sealed and the trap catch transported back to the laboratory. The flight intercept trap catch was preserved in 70% alcohol for later identification. A cotton pad soaked with ethyl acetate was dropped into the light traps, which were then re-sealed and left for nine hours, after which the insects were transferred into sample bottles. Care was taken to store moths separately from delicate flies to prevent scale loss and damage to the fly wing membranes. The sweep net catches were collected in sample bottles. The insect catches were then frozen for identification at a later date.

5.2.3. Insect identification

Insects were identified to family and the moths were identified to species (Colyer & Hammond 1968; Unwin 1981; Harde 1984; Sterny 1997; Skinner 1998). Refer to appendix 8.2 for a full list of moth species captured on both farms. Where identification

was not possible due to missing parts, or where there was any uncertainty, the insects were classified as 'Not Identifiable' and left out of the analysis of diversity. Eighteen insect families known to be important components of bat diet (Vaughan 1997) were chosen for detailed analysis; each of these key families is a major component of the diet of at least one bat species in the UK (Table 5.1).

Bats were grouped by feeding trait or as one of the six species with a BAP. Insect families were grouped by order to investigate the relationship between the activity of these bat groups, determined by bat pass number, and the abundance of their main food groups. Relationships between prey type specialists and key insect families were also investigated. The combined abundance of Lepidoptera and Diptera were also tested for relationships with all the bat groups as many bat species take both types of insect as part of their diet.

After counting and identification, the dry masses of total trap catches within habitats on organic and conventional farms was determined. The total catch for each habitat on each farm was placed in an oven at 50°C, and left for up to 40 hours. The mass was measured at intervals until it was constant, after which the final dry mass was determined.

The use of higher taxa, such as family richness, as a surrogate for species richness has been validated in a number of studies (Balmford *et al.* 1996a, b; Hughes *et al.* 2000). The relationship between species richness and family richness was explored for moths.

5.2.4. Statistical methods

The differences between farm types were analysed using paired-sample *t* tests; data were log transformed ($\log_{10}(X+1)$) if necessary to achieve normality in the differences

(Zar 1999). These statistical analyses were performed using Minitab version 13 (Ryan & Joiner 1994). To evaluate the predictive power of using family as an indicator for species richness linear regression models were used on the log transformed data ($\log_{10}$ (X+1)) for both farm types separately. Shannon-Weiner diversity indices were calculated to test for differences in diversity between farm type. The differences between farm types in the number of insects belonging to key insect families and in species richness were analysed using the Wilcoxon paired-sample test, as the data were not normally distributed (Zar 1999).

5.3 Results

7598 insects were captured in 240 hours of sampling time; 7548 were identified to family (Table 5.2) and 1189 of 1239 moths were identified to species. The unidentified moths belonged to the pug family, notoriously difficult to classify to species.

When organic and conventional farms were considered together there was no correlation between number of insects and hedgerow height or wind-speed, but a significant correlation was found between insect abundance and temperature (Spearman's coefficient correlation, r_s=0.286, d.f.=46, P=0.049).

Table 5.1. Key insect families important in bat diets in Britain (families that make up over 10% of diet).

Insect order	Family	BAP species[a]						Other species[b]
		R.f	*R.h*	*M.b*	*B.b*	*P.p*	*P.py*	
Coleoptera	Carabidae	✓						*N.n, E.s*
	Scarabaeidae	✓						*N.n, N.l, E.s*
Diptera	Tipulidae		✓	✓		✓	✓	*M.br, M.m, M.n, M.d, N.l, N.n, P.a, P.au*
	Culicidae		✓	✓				*N.l, N.n*
	Anisopodidae		✓	✓				*M.m, M.br, N.n, P.a*
	Sciaridae							*M.n, N.l*
	Chironomidae		✓			✓	✓	*M.br, M.d, P.n, N.l, N.n, P.a*
	Dolichopoidae							*M.n, N.l*
	Ceratopogonidae		✓			✓	✓	*N.l*
	Psychodidae					✓	✓	*M.m*
Lepidoptera	Pyralidae							*N.l, P.a*
	Arctiidae				✓			*P.a*
	Noctuidae	✓		✓				*N.l, P.a, P.au*
	Geometridae	✓						*N.l, P.a, P.au*
Trichoptera	Limnephilidae	✓				✓		*M.d*
	Brachycentridae	✓	✓			✓	✓	*M.d*
	Molannidae	✓	✓			✓	✓	*M.d*
	Beraeidae	✓	✓			✓	✓	**M.d**

[a]Bat species that have biodiversity action plans (BAPs) in the United Kingdom.

[b] *Rhinolophus ferrumequinum (R.f), R. hipposideros (R.h), M. bechsteinii (M.b), M. nattereri (M.n), M. mystacinus (M.m), M. brandtii (M.br), Myotis daubentonii (M.d), Barbastella barbastellus (B.b), Pipistrellus pipistrellus (P.p), P.pygmaeus (P.py), P. nathusii (P.n), N. leisleri (N.l), Nyctalus noctula (N.n), Eptesicus serotinus (E.s), Plecotus auritus (P.au), Plecotus austriacus (P.a).*

5.3.1. Insect abundance

More insects were captured on organic than on conventional farms (t=6.55, d.f.=23, P<0.000; Fig. 5.1). When analysed by habitat type, significantly higher insect abundance was found on organic pastural and water habitats compared with the same habitats on conventional farms (Table 5.3).

Insect dry mass was significantly higher on organic farms than on conventional farms (t=2.11, d.f.=23, P= 0.046; Fig. 5.2). Within individual habitats, insect dry mass was significantly higher in pastural (t=3.97, d.f.=20, P= 0.001) and woodland habitats (t=2.94, d.f.=9, P= 0.017) than on the same habitats on conventional farms.

5.3.2. Insect diversity

There was a significant correlation between moth species richness and moth family richness in both farm types (Fig. 5.3; Spearman's coefficient correlation, r_s=0.831, n=48, P<0.001). The predictive power of both models was high (linear regressions: organic farms: F=107.3, df=1, 22, r^2=0.83, p=0.000; conventional farms: F=61.9, df=1, 22, r^2=0.74, p=0.000). Therefore, family richness as an indicator of species richness, was used as advocated by Balmford et al. 1996*a*, *b*. The difference in the total number of insect families between farm type was significant, with a higher family richness (indicating higher species richness) on organic than on conventional farms (Wilcoxon paired-sample test, Z=-3.045, d.f.=23, P=0.002; Fig. 5.4). Moth species richness was also significantly higher on organic than on conventional farms (Wilcoxon paired-sample test, Z=-3.360, d.f.=23, P=0.001), as was moth species diversity (Wilcoxon paired-sample test, Z=-2.277, d.f.=23, P=0.023).

Table 5.2. List of insect families identified with mean numbers of insects captured in each farm type.

Family[a]	Organic farm Mean ±SEM[b]	Conventional farm Mean ±SEM[b]	Family[a]	Organic farm Mean ±SEM[b]	Conventional farm Mean ±SEM[b]
Forficulidae	0.42±0.16	0.17±0.10	Tipulidae[c]	3.50±5.20	2.35±0.94
Berytidae	0.21±0.08	0.04±0.04	Trichoceridae	3.02±0.66	1.82±0.21
Anthocoridae	1.03±0.49	1.25±0.26	Culicidae[c]	1.67±1.20	0.44±0.24
Corixidae	11.41±6.13	4.15±0.44	Anisopodidae[c]	3.95±0.95	6.04±1.97
Cercopidae	1.13±0.28	0.33±0.13	Scatopsidae	1.62±0.30	5.02±2.08
Cicadellidae	0.25±0.10	0.29±0.11	Cecidomyiidae	15.63±3.63	8.32±2.21
Aphididae	0.04±0.04	0.17±0.08	Sciaridae[c]	6.13±1.94	8.50±5.38
Carabidae[c]	1.04±0.30 *	0.29±0.42	Chironomidae[c]	67.17±15.55 **	33.16±13.09
Hydrophilidae	1.65±0.69	1.22±0.16	Blephariceridae	0.25±0.11	0.04±0.04
Byrrhidae	0.29±0.11	0	Phoridae	1.79±0.68	0.17±0.10
Scarabaeidae[c]	1.65±0.61	0.35±0.17	Lonchopteridae	3.86±1.05	1.06±0.19
Chrysomelidae	2.31±0.70	0.87±0.18	Dolichopodidae[c]	0.83±0.29	0.63±0.22
Hydraenidae	2.23±1.28	1.45±0.63	Certatopogonidae[c]	1.69±0.10	2.68±2.37
Curculionidae	1.04±0.33	1.04±0.23	Psychodidae[c]	13.15±3.08 **	5.86±1.69
Syrphidae	0.04±0.04	0.17±0.08	Limnephilidae[c]	2.44±1.39	1.58±0.60
Lauxaniidae	0.17±0.08	0.13±0.07	Brachycentridae[c]	0.29±0.19	0.04±0.04
Sepsidae	2.45±0.61	3.15±0.98	Hydropsychidae	0.29±0.14	0
Opomyzidae	0.42±0.13	1.04±0.27	Polycentropidae	0.42±0.20	0
Scathophagidae	1.93±0.82	1.22±0.27	Psychomyiidae	0.71±0.22	0
Muscidae	0.46±0.18	0.13±0.09	Odontoceridae	1.20±0.33	0.13±0.07
Mycetophilidae	0.46±0.33	0.33±0.12	Glossomatidae	2.43±0.65	2.11±0.49
Ptychopteridae	0.17±0.10	0.13±0.07	Molannidae[c]	1.00±0.56	0.33±0.22
Cochylidae	0.33±0.12	0.29±0.09	Beraeidae[c]	0.75±0.30	0.61±0.20
Pyralidae[c]	1.96±0.92	2.33±0.56	Cynipidae	0.29±0.18	0.04±0.04
Micropterigidae	3.96±1.06	2.12±0.42	Ichneumonidae	1.85±0.46	1.72±0.41
Eriocraniidae	0.20±0.10	0.17±0.08	Formicidae	0.38±0.12	0.38±0.13
Yponomeutidae	0.88±0.28	0.58±0.16			
Arctiidae[c]	2.36±1.25	1.22±0.92			
Noctuidae[c]	17.05±4.68 **	8.35±2.10			
Lasiocampidae	0.17±0.10	0.17±0.10			
Geometridae[c]	4.04±1.12 **	2.15±0.81			

[a]Only families with total numbers of five and above are shown.
[b]SEM=standard error of the mean.
[c]Key insect families important to the diet of bats in the United Kingdom. Probability: *p<0.05, **p<0.01. Degrees of freedom = 23.

Fig. 5.1 Differences in the numbers of insects per farm pair (organic - conventional). Black bars indicate more insects on organic farms, white bars more insects on conventional farms (24 pairs).

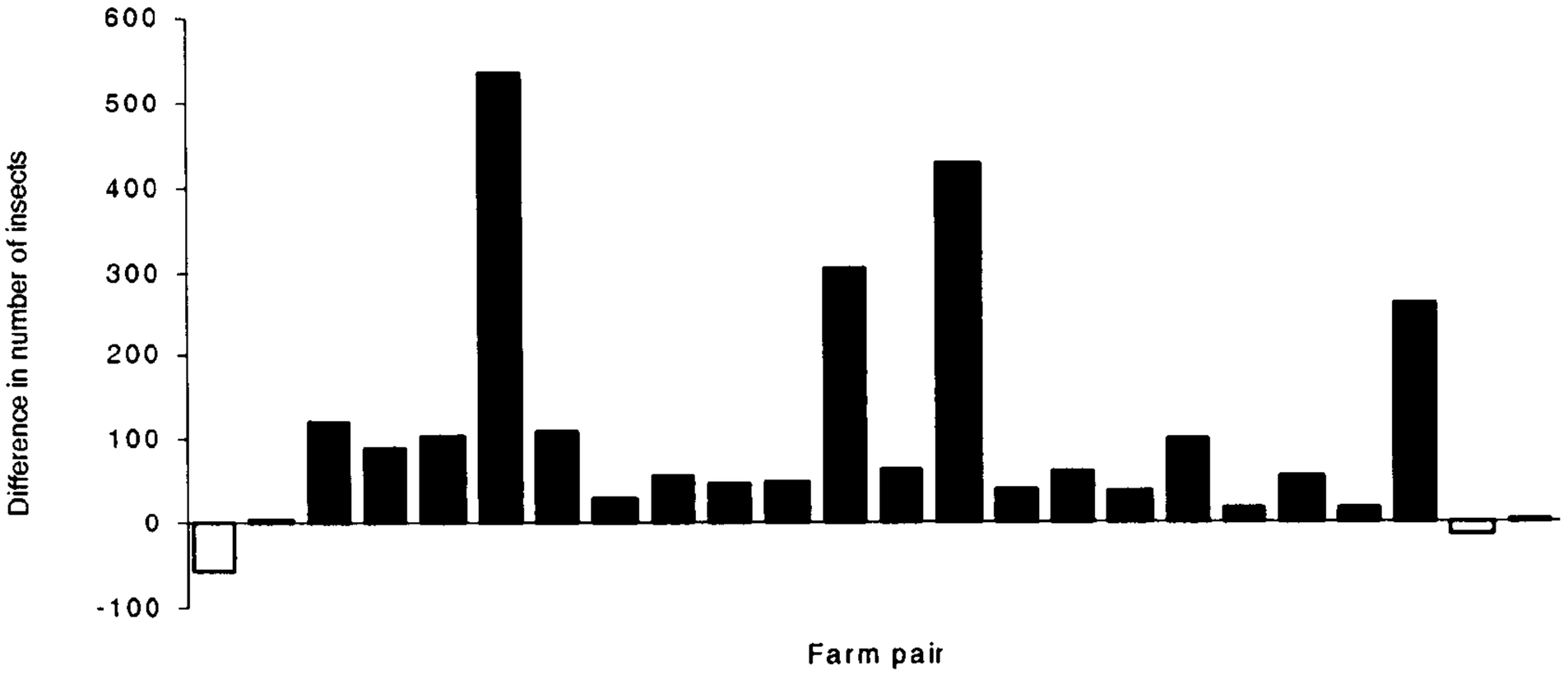


Fig. 5.2 Differences in total insect dry mass per farm pair (organic-conventional). Black bars indicate higher dry mass on organic farms, white bars higher dry mass on conventional farms (24 pairs).

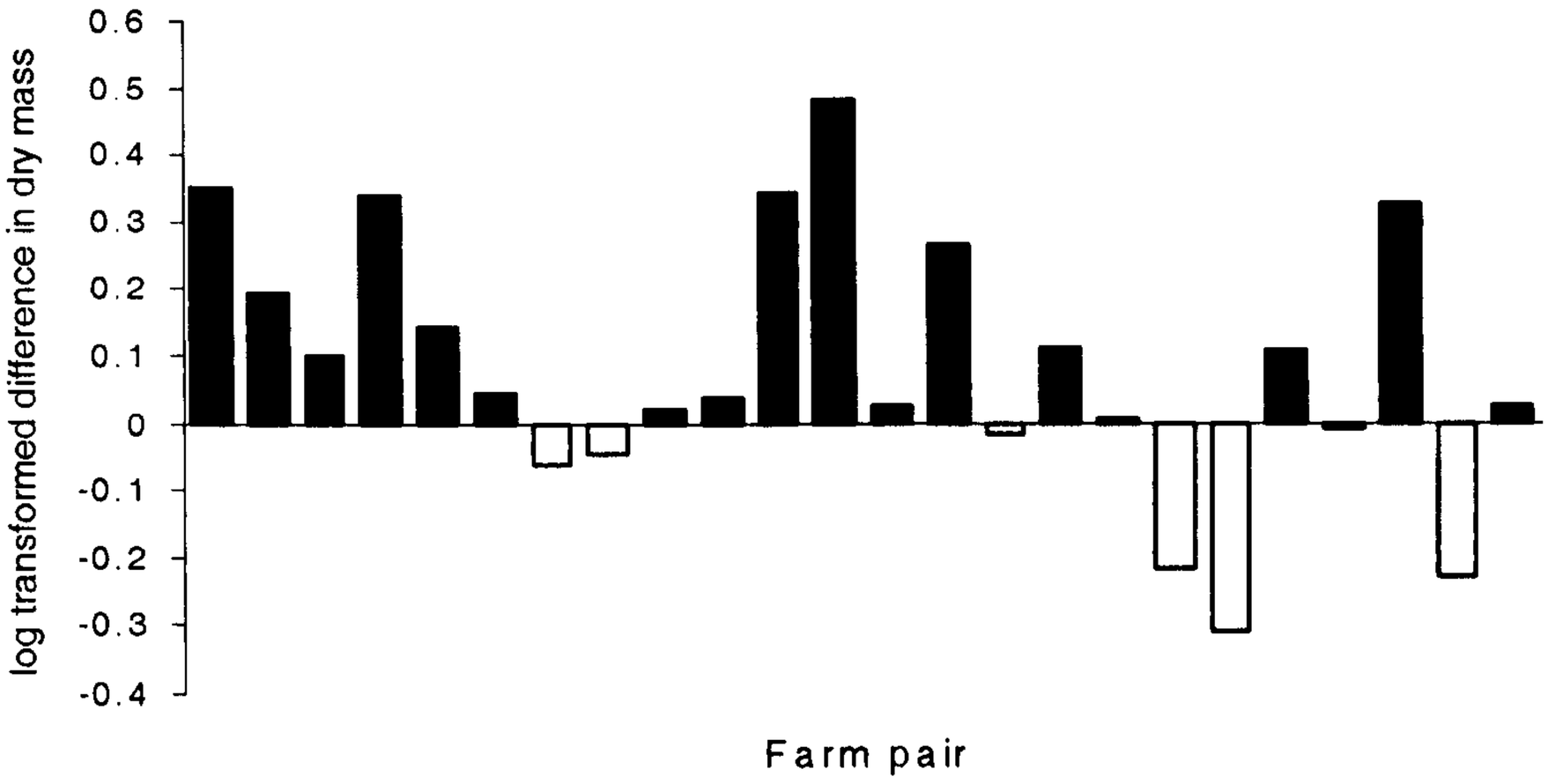


Table 5.3 Statistical significance of differences in insect abundance within habitats per farm pair. A significant result indicates a higher abundance on habitats in organic farms.

Habitat	***n***	***t***	***P***
Pasture	21	4.35	<0.001
Arable	8	1.54	0.167
Water	8	3.03	0.019
Woodland	10	1.85	0.097

Fig. 5.3 Correlation between moth species and moth family richness.

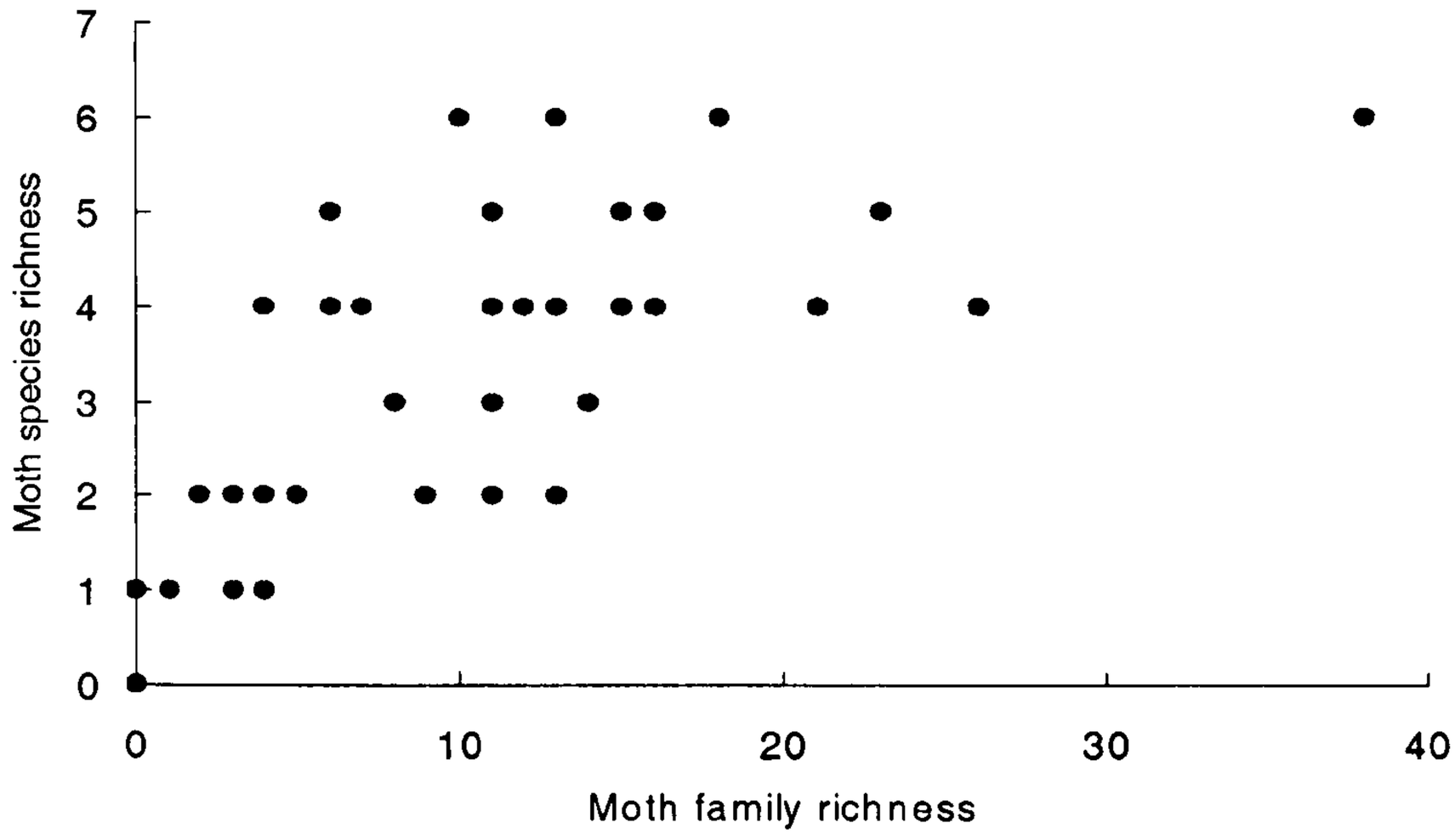
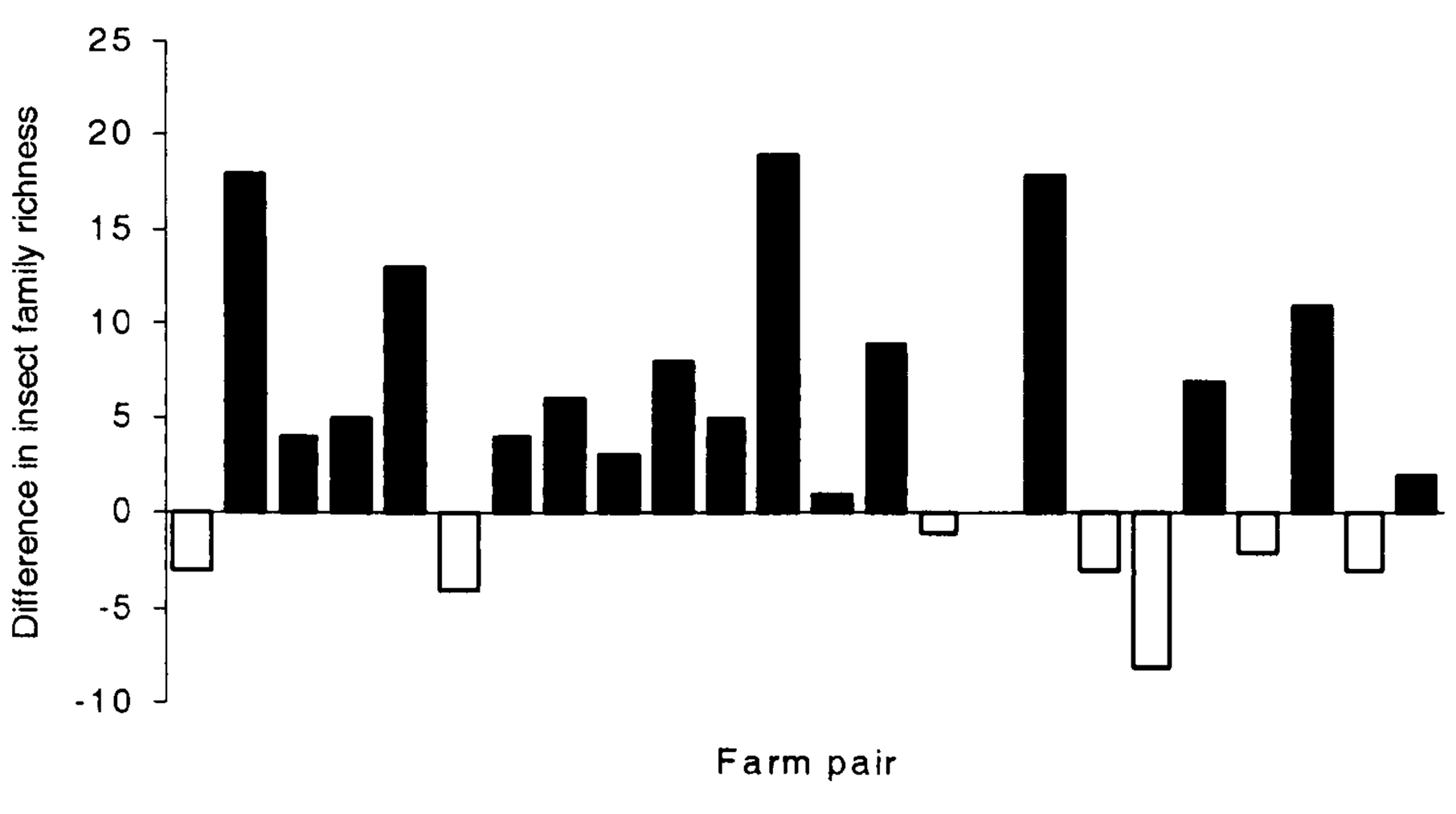


Fig. 5.4 The differences in insect family richness per farm pair (organic –conventional). Black bars indicate higher family richness on organic farms, white bars higher family richness on conventional farms (24 pairs).



5.3.3. Key insect family abundance

Of the 18 key insect families, the numbers of insects belonging to five were captured in significantly higher numbers on organic than on conventional farms (Table 5.4). These were two lepidopteran families, two dipteran families and a coleopteran family. There

were significant differences in the abundance of insects belonging to the key families in different habitats between farm types (Table 5.4). Over water habitats the abundance of Psychodidae was significantly higher on organic farms than on conventional farms. In woodland habitats the abundance of the dipteran family Chironomidae was significantly higher on organic farms. Over pastural habitats, Noctuidae, Geometridae, Arctiidae, Psychodidae, Chironomidae, Scarabaeidae, and Carabidae were all captured in significantly higher numbers on organic farms than on conventional farms. There was no significant difference in key groups between farm type within arable habitats. No key insect families were found to be significantly more abundant on conventional farms.

5.3.4. Relationship between key insect family differences and bat activity

Table 5.5 represents an overview of the activity of those bat species most affected by agricultural intensification (see Chapter 4; Wickramasinghe *et al.* 2003), and the abundance of insect families most commonly eaten by those species. Certain *Myotis* bat species, and the dipteran families they eat, are both significantly more abundant on organic farms overall and within organic water habitats than on conventional farmland habitats. Specifically, there was significantly more Myotis daubentonii and M. bechsteinii on organic farms annd significantly more M. bechsteinii and M. brandtii over water bodies on organic farms (Ch 4.). This is consistent with Psychodidae and Chironomidae being more abundant on organic farms, with Psychodidae captured in higher numbers in pastural habitats and water bodies on organic farms than on conventional farms, and Chironomidae captured in higher numbers in pastural and woodland habitats on organic farms than on conventional farms.

There was no relationship between total bat activity and total insect abundance (Spearman's coefficient correlation, r_s=0.175, n=48, P=0.235). A significant correlation was found between the activity of bats that mainly eat Lepidoptera and the abundance of

Lepidoptera (P=0.044). This group of bats was also significantly correlated with Lepidoptera and Diptera combined (P=0.016; Table 5.6). The significance of the relationship between the dipteran predators and the abundance of Diptera and the abundance of Lepidoptera and Diptera combined was close to <0.05, as was the relationship between the activity of BAP species of bat and the combined abundance of Lepidoptera and Diptera (Table 5.6). The relationship between dipteran predators and Psychodidae abundance was negative but all the other relationships were positive.

Table 5.4. Mean ± standard error of the mean for numbers of insects captured in each habitat and statistical difference in abundance of key insect families between farm pairs.

Family[a]	Pasture:		Arable:		Woodland:		Water:	
	Organic	Conventional	Organic	Conventional	Organic	Conventional	Organic	Conventional
Carabidae	0.76±0.25 *	0.23±0.11	0.43±0.43	0	0.18±0.18	0.09±0.09	0.57±0.30	0.14±0.14
Scarabaeidae	0.43±0.18 *	0.05±0.05	2.71±1.49	0	0.18±0.12	0.27±0.14	1.43±0.57	0.57±0.37
Tipulidae	1.86±0.72	0.86±0.34	0.29±0.18	0.71±0.42	3.73±1.96	1.82±0.76	0.29±0.29	0.43±0.20
Culicidae	1.76±1.37	0.38±0.25	0.14±0.14	0	0.18±0.12	0.18±0.12	0	0
Anisopodidae	2.09±0.54	2.28±0.58	1.14±0.63	6.14±4.71	3.18±1.12	3.91±2.19	1.14±0.63	1.57±0.97
Sciaridae	3.52±1.09	4.95±1.56	2.57±1.23	5.29±4.03	0.63±0.54	4.45±6.19	6.86±5.78	2.00±0.95
Chironomidae	36.24±8.59 **	15.33±3.43	11.71±2.57	46.85±36.94	18.00±6.18 *	4.27±1.51	81.57±32.34	14.14±3.19
Dolichopodidae	0.48±0.19	0.24±0.11	0	0.14±0.14	0.55±0.39	0.55±0.31	0.57±0.30	0.14±0.14
Ceratopogonidae	1.47±1.06	0.33±0.23	0.29±0.29	8.14±8.14	0.45±0.37	0	0.43±0.43	0
Psychodidae	5.86±1.66 *	3.57±1.18	3.00±1.48	2.57±1.23	5.18±3.08	1.81±0.74	16.14±7.15 *	4.00±3.00
Pyralidae	1.14±0.58	1.52±0.54	1.43±1.43	1.71±0.94	0.36±0.20	0.45±0.21	0.57±0.43	1.00±0.58
Arctiidae	1.28±0.55 *	0.10±0.10	1.57±1.06	1.43±0.97	1.55±1.45	1.36±1.26	0.29±0.18	0.29±0.18
Noctuidae	10.71±3.01 **	4.61±1.00	12.43±5.31	4.43±2.02	6.09±1.01	4.81±1.74	4.29±2.54	2.71±1.22
Geometridae	2.24±0.54 **	0.76±0.28	1.86±0.96	2.14±0.91	2.27±1.20	1.45±0.80	1.71±0.36	0.71±0.57
Limnephilidae	0.42±0.20	0.47±0.18	0.43±0.43	1.00±0.58	3.09±2.71	0.64±0.36	1.71±1.23	2.00±1.00
Brachycentridae	0.33±0.21	0.05±0.05	0	0	0	0	0	0
Molannidae	0.53±0.53	0.05±0.05	0.14±0.14	0	0.72±0.55	0.45±0.45	0.57±0.57	0.23±0.23
Beraeidae	0.52±0.43	0	0.57±0.57	0.43±0.30	0	0	0.43±0.30	1.57±0.90
Unidentified	5	2	8	4	2	5	1	5

[a]Key insect families are families important in the diets of British bats. Probability: *$p<0.05$, **$p<0.01$. A significant result indicates higher abundance on organic habitats. In no family was there a significantly higher insect abundance on conventional habitats. Degrees of freedom: pasture 20, woodland 9, water 7, arable 7 (Wilcoxon paired-sample test).

Table 5.5 The activity of those bat species most affected by agricultural intensification and the insect families they commonly eat. *R.h (Rhinolophus hipposideros), M.d (Myotis daubentonii), M.be (M. bechsteinii), M.br (M. brandtii), M.mys (M. mystacinus).* P (pasture), A (arable), WO (woodland), WA (water).

✓ Denotes >50% of bat activity or insect abundance within habitats on organic farms.

• denotes <50% of bat activity or insect abundance within habitats on organic farms.

* denotes significantly higher bat activity or insect abundance within habitats on organic farms than on conventional farms, P<0.05.

** P<0.01

Habitats						Habitats					
Bat species						Insect Families					
	Total	P	A	WO	WA		Total	P	A	WO	WA
R.h		•	•	✓		Psychodidae	**	*•	•	•	*•
M.d	*	•	•	•	✓	Tipulidae		•	•	✓	•
M.be					✓ *	Chironomidae	**	•**	•	*•	•
M.br	*	•	•	•	✓ *						
M.mys			•	•	✓						

Table 5.6 Correlations between bats grouped by feeding trait and total abundance of insects belonging to order or key insect family.

Bat group	Food group/key insect family	*n*	Correlation coefficient (r_s)	*P*
Lepidopteran Predators	Lepidoptera	48	0.239	0.044
	Noctuidae	48	0.139	0.347
	Geometridae	48	0.276	0.051
	Arctiidae	48	0.223	0.128
	Lepidoptera	48	0.346	0.016
	Diptera (combined)			
Dipteran Predators	Diptera	48	0.251	0.081
	Psychodidae	48	-0.073	0.621
	Chironomidae	48	0.161	0.274
	Lepidoptera	48	0.279	0.051
	Diptera (combined)			
BAP species	Lepidoptera	48	0.210	0.152
	Diptera	48	0.220	0.133
	Lepidoptera	48	0.268	0.064
	Diptera (combined)			

5.4 Discussion

5.4.1. Changes in insect abundance

The paired experimental design used standardized for as many variables as possible between farms in a pair with the exception of farm type, the main difference being the presence or absence of agrochemicals.

In this study, insect family richness was used as an indicator of species richness. Balmford et al. (1996*a*, *b*) state that even when relationships between higher taxa and numbers of species are significant, they vary in strength and the prediction of absolute species richness may be low. The use of higher taxa in this study was validated by the high predictive power resulting from the linear regression models for both farm types. However, variation in predictive power may not be important if the goal of higher taxa surveys is to rank the relative richness of sites (Balmford et al. 1996*a*, *b*).

The null hypotheses that insect abundance, mass and species richness are the same on organic farms and conventional farms can be rejected, as significantly higher nocturnal and crepuscular aerial insect abundance, mass and species diversity were found on organic farms than on conventional farms. The reason for these differences is most likely to be the use of agrochemicals on conventional farms. Pesticides have been shown to reduce insect numbers both of target species and, through spray drift, of non-target species in unsprayed headlands (Sotherton 1991; Chiverton & Sotherton 1991; de Snoo 1999). In the UK, antihelminthic drugs such as avermectin, used for cattle and sheep, may reduce insect fauna in dung (Strong 1992), especially scarabid and geotrupid dung beetles, which are a dominant part of the diet of *R. ferrumequinum*, *N. noctula* and *E. serotinus* (Hutson *et al.* 2001). Carabid populations of organic fields have been shown to be significantly richer in species and abundance than in low-input integrated crop management farmed plots (Pfiffner & Luka 2003).

Other studies have shown that insect diversity is generally lower in more intensively managed fields (Carcamo *et al.* 1995; di Guilio *et al.* 2001; Benton *et al.* 2002). In addition to direct insecticidal effects, herbicides used in intensively managed farms also have indirect effects on invertebrate populations by removing their food plants (Moreby & Southway 1999; Robinson & Sutherland 2002). Insect species richness is positively related to plant species richness and plant functional group richness (Strong *et al.* 1984; Haddad *et al.* 2001). As organic standards prohibit the use of herbicides, the quality of habitats found on this farm type is high in terms of habitat structure and plant diversity compared to conventional farms (Mansvelt *et al.* 1998).

The concept of landscapes as complex mosaics of habitats varying in quality with respect to different groups of organisms has been the subject of a number of recent studies (Weins 1995; Gascon *et al.* 1999; Ricketts *et al.* 2001). Patches of habitat with varying quality are likely to underlie the differences found in insect abundance between farm types. Synthetic chemicals and nutrients such as nitrogen and phosphorous are major contributors to aquatic pollution (Angier *et al.* 2002). Excess phosphorous in a system primarily affects aquatic life, excess nitrogen affects aquatic life and can be damaging to human health (Hapeman 2002). An increase in organic pollution from manure run-off results in a gradual decrease in the oxygen content of the water, which can affect sensitive organisms such as trichopteran larvae and Plecoptera (Mason 2002), although the actual impact depends on the severity of oxygen depletion. As the oxygen content gradually increases, the numbers of chironomids and other insects increase (Mason 2002), which may explain the higher activity of such insects on organic farms where organic run-off is likely to occur. The significantly higher insect abundance over water bodies on organic farms was probably due to good water quality i.e. the absence of agrochemical pollutants, and the fact that almost all of these sites were surrounded by

trees or bushes, thereby providing shelter for emerging aquatic insects and dead leaf beds important for other groups of insects. Racey *et al.* 1998 showed that Psychodidae abundance was highest over a eutrophic river compared to an oligotrophic one.

Although the main difference between organic and conventional farms is the use of synthetic chemicals, there are undoubtedly mechanisms other than agrochemical use linking intensive farming with the reduction of insects. Kirby (2001) identified habitat continuity and structural variation as the two most important factors in maintaining insect populations at a site. Vegetation structure at the microhabitat level is also important for insect communities and a reduction in grazing intensity, for example, has been shown to enhance insect diversity (Kruess & Tscharntke 2002). Insect densities are generally higher nearer vertical landscape elements than in open areas (Lewis 1970; Lewis & Dibley 1970).

5.4.2. Implications for bat foraging

In this Chapter, the impact of agricultural intensification on key families of insects important to bat diet overall, and within habitat types, was evaluated. Habitat type is known to be an important factor for specific insect groups (Huges *et al.* 2000). As well as the clear difference in total insect number between farm type, there was also a greater abundance of insects belonging to five key insect families on organic farms. These included lepidopteran, coleopteran and dipteran families.

The high numbers of larger insects such as Lepidoptera and Coleoptera on organic farms explains the higher dry mass measurements found on this farm type both overall and within pastural habitats. A study comparing trends in moth numbers in different habitats showed a general decline in farmland populations, but little change in

woodland populations (Woiwood & Harrington 1994); the results of this Chapter support the hypothesis that agricultural intensification has contributed to this decline.

Correlations between the activity of bats and the abundance of their prey were investigated. By comparing the activity of bat species most affected by agricultural intensification with the abundance of those insect families most commonly eaten by these bats, a number of key insect families were found to be significantly more abundant on organic farms and this was correlated with higher activity levels of the bat species that preyed on them. Although total bat activity was not correlated with total insect abundance, the activity of bats whose diet consisted mainly of Lepidoptera was correlated with the abundance of both Lepidoptera and with the abundance of the Lepidoptera and Diptera combined. Other relationships between prey abundance and bat predator activity were positive and close to significance. These findings suggest that as bats are probably resource limited, increasing the numbers of key families of insect will increase the numbers of bats and other predators.

5.5 Summary

- Higher total insect abundance was found on organic farms overall and within organic pastural and water habitats
- Total insect dry mass was higher on organic farms overall and within organic pastural and woodland habitats
- Total insect species richness was higher on organic farms
- Many key families important to bat diet had higher insect abundance on organic farms overall and some families had higher numbers of insects within organic woodland pastural and water habitats

- Correlations between the activity of bat and the abundance of the insect prey support the hypothesis that as bats are likely to be resource limited, a reduction in prey will result in a reduction in bat activity

In the next Chapter, I bring together the main findings from previous Chapters and discuss them in terms of their contribution to acoustic survey methods and their conservation relevance. I also highlight points for further study.

CHAPTER 6

CONCLUSION

6.1 Outcome of the study

6.1.1. Acoustic monitoring of bat populations

Populations of aerial-feeding bats are more conveniently monitored via an acoustic method than by netting in open environments. There are various techniques employed by researchers in order to record and analyse echolocation calls and different methods result in differing call descriptions and, sometimes, incorrect species classification. The aims of Chapters two and three were to compare the efficiency of the two main frequency reduction techniques, and to test existing identification methods. In Chapter two, I showed that both the technique used to reduce the high frequency calls from a bat to a lower frequency as well as the method used to transform the calls to analyse them in the frequency time domain can significantly affect call descriptions. In Chapter three I went on to show that the discriminating capabilities of ANN and DFA on FD calls were significantly lower than on TE calls. The results of Chapters two and three highlight the differences generated by two of the most widely used techniques and emphasises the importance of tailoring the recording methods to suit the goal of the research. The quantitative and objective nature of the direct sampling system coupled with the use of ANNs for species identification, as used in this study, is an advance on the techniques used in previous studies (Neefus & Krusic 1995; Walsh & Harris 1996 a, b; Parsons 1997). Accurate surveys of general bat activity can be achieved through relatively inexpensive equipment. However, when assessing the activity of species in order to gain information on habitat use or habitat preference, accurate

identification is crucial. Acoustic identification methods should be objective and quantitative so they can be repeated by other researchers and used to generate a reliable database of species information.

6.1.2. Impact of agricultural intensification on biodiversity

The aim of Chapters four and five was to investigate the impact of agricultural intensification on British bat populations and their insect prey. The findings reported in Chapter four, that bat activity was significantly higher in organic farms than conventional farms add to the growing body of published data on the wildlife benefits of organic farming (Feber *et al.* 1997; Chamberlain *et al.* 1999; Beecher *et al.* 2002), and highlight the position of bats as bioindicators and victims of agricultural change.

As British bats are insectivorous, declines in insect abundance as a result of agricultural intensification are likely to have serious implications for bat foraging. This was addressed in Chapter five in which nocturnal insect abundance and species richness was found to be lower in conventional than on organic farms. The reduction in insect abundance included reductions in insect species known to be key dietary constituents of bats. Invertebrates have very specific habitat requirements with some showing host specificity, and without the correct plant diversity and habitat structure insect families can decline rapidly. The intensification process simplifies the landscape by removing non-crop habitats important to a number of insect families.

Changes in land use through agricultural intensification have reduced resource abundance for bats, and also reduced the stability and predictability of such food resources. As bat populations are likely to be resource limited (Bonaccorso 1979; Findley 1993), the data support the hypothesis that agricultural intensification has been a factor in the

reduction in the numbers of key dietary components for bats and that this adverse effect on the food web has led to reduced bat activity and altered species composition on conventional farms.

6.2 Further work

A result warranting further investigation was the higher bat activity and bat species composition over water habitats on organic than on conventional farms. This was coupled with significant differences in insect abundance adjacent to this habitat type suggesting a link between bat activity and water quality. Further work on the levels and type of pollutants and/or sources of organic enrichment present in conventional water habitats, and how these pollutants affect particular insect groups, will be beneficial to both insect and bat conservation.

This study focused on specific habitat types within farms. Work looking into the structural differences within each habitat between farm type would provide further information on the specific habitat needs of various insects, and lead to more detailed habitat management guidelines that would ultimately benefit bat populations.

The issues of habitat fragmentation and habitat patch isolation are important for bat conservation. At a large spatial scale, agricultural intensification alters the landscape by fragmenting habitats, reducing linear features and decreasing connectivity. Habitat patches are part of a landscape mosaic and the presence of a given species in a patch may be a function not only of patch size and isolation, but also of the kind of neighbouring habitat (Andrén 1994). The organic farms studied were in effect isolated patches in an essentially conventionally farmed landscape. Bats may only be able to reach prime foraging habitats in isolated patches, if the overall landscape connectivity allows this through suitable

surrounding habitats, such as quality hedgerows and woodland edges acting as flight corridors.

Through extensive GIS mapping of farms, their habitats and the surrounding area, one could investigate the effect of agricultural intensification on landscape connectivity, and how at this larger spatial scale, connectivity and surrounding landscape features can influence bat activity.

In light of the presented data on the adverse effects of agricultural intensification on British bat populations, the implications of the widespread use of GM agriculture is cause for concern. Careful research into the effects of pest resistant crops on the insect fauna and the landscape effects of such agriculture is needed. There is the possibility that further simplification of the landscape and reduction of insect numbers, through GM agriculture, could make the current situation for British bats dramatically worse.

6.3 Contribution to bat conservation

Agricultural intensification is a global phenomenon and impacts biodiversity on a vast scale. Understanding the mechanisms behind species declines is the first step in trying to reverse them. The results presented in this thesis are an important contribution to the elucidation of causes of declining bat populations as well as to the growing information on the impact of organic farming on biodiversity in temperate agriculture. The impact of intensification on bat prey also highlights the adverse effect it has on food webs, which can be a driving factor in species population declines.

The findings described in this thesis emphasise the importance of habitat management in farmland for bat conservation. In terms of restoring bat numbers, a less intensive system of farming will benefit bats by improving the quality of flight paths and

foraging habitat. Furthermore, the data suggest that managing farms to maximise insect abundance, especially that of key insect families, by maintaining diverse and structurally varied habitats and reducing agrochemical use, will benefit bat populations, and may aid in reversing population declines.

Recommendations such as the reduction of agrochemical use and management of particular habitats, for example, tall herb-rich hedgerows, water habitats and non-crop habitats, will not only benefit bat populations, by maintaining a healthy insect prey base, but will also benefit a number of other declining farmland species that rely on such habitats and food sources for survival.

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APPENDIX 1

FARM DETAILS

Pair No.**	Farm Type*	Farm Name	OS Coordinates	Businesses Type ***	Farm Area (Ha)	Habitats Sampled	Type of Water Body Sampled
1	O	Little Mill	SO 426173	Mixed	11.85	Pasture, Woodland	-
1	C	Farr	SO 432176	Cattle and sheep	18.53	Pasture, Woodland	-
2	O	Radford	ST 665578	Mixed	27.62	Pasture, Arable	-
2	C	Sheephouse	ST 686585	Mixed	68.92	Pasture, Arable	-
3	O	Oakhall	ST 669939	Mixed	31.66	Pasture	-
3	C	Glen	ST 677942	Cattle	34.44	Pasture	-
4	O	Hamfield	ST 664994	Cattle and sheep	26.21	Pasture, Woodland	-
4	C	Woodland	ST 662983	Mixed	55.54	Pasture, Woodland	-
5	O	Shrubb/Castle Combe	ST 835766	Mixed	59.72	Pasture, Arable, Woodland	-
5	C	Upper Castle Combe	ST 849774	Mixed	54.06	Pasture, Arable, Woodland	-

6	O	New House	SO 746042	Mixed	16.25	Pasture, Arable, Water	Stream
6	C	Manor	SO 747081	Mixed	28.72	Pasture, Arable, Water	Canal
7	O	Hookergate	SU 044832	Mixed	54.78	Pasture, Water	Brook/Stream
7	C	Vines	SU 034829	Cattle	30.36	Pasture, Water	Brook/Stream
8	O	Firlands	SU 643666	Sheep	34.95	Pasture, Woodland	-
8	C	Abbots	SU 647677	Mixed	36.12	Pasture, Woodland	-
9	O	Westlodge	SU 651779	Mixed	13.60	Pasture	-
9	C	Newhouse	SU 651789	Mixed	34.31	Pasture	-
10	O	Butlers	SU 640789	Mixed	56.46	Pasture. Arable. Woodland	-
10	C	Hammonds	SU 653834	Mixed	82.95	Pasture. Arable. Woodland	-
11	O	Waltham Place	SU 856774	Mixed	65.18	Pasture. Woodland. Water	Ponds
11	C	Shottersbrook	SU 841773	Cattle and sheep	117.19	Pasture. Woodland. Water	Ponds
12	O	Floodgates	ST 678987	Cattle	39.40	Pasture. Water	Brook/Stream

12	C	Oakleaze	ST 694971	Mixed	93.88	Pasture, Water	Brook
13	O	Woodford Green	ST 693962	Cattle	43.98	Pasture	-
13	C	Swanley	ST 701964	Cattle	55.38	Pasture	-
14	O	Brickhouse	ST 649962	Cattle	56.38	Pasture, Water	Stream
14	C	Worldsend	ST 651975	Mixed	50.68	Pasture, Water	Stream
15	O	Green	ST 653934	Cattle	24.65	Pasture, Water	Stream/Ponds
15	C	Lodge	ST 649934	Mixed	24.59	Pasture, Water	Stream/Ponds
16	O	Hill House	ST 550666	Sheep	24.52	Pasture, Woodland	-
16	C	Castle	ST 547669	Sheep	25.21	Pasture, Woodland	-
17	O	Brownsmill	ST 683982	Cattle	62.33	Woodland, Water	Stream
17	C	Alkington	ST 694983	Cattle	68.80	Woodland, Water	Stream
18	O	Chilly Hill House	ST 564627	Cattle and sheep	29.10	Pasture	-
18	C	Pagans Hill	ST 559624	Mixed	32.81	Pasture	-

19	O	Norwood	ST 778571	Mixed	133.81	Pasture, Arable	-
19	C	Highchurch	ST 742539	Mixed	87.05	Pasture, Arable	-
20	O	Whitfield	ST 684916	Mixed	71.66	Pasture, Woodland	-
20	C	Abbotside	ST 687908	Cattle and sheep	46.43	Pasture, Woodland	-
21	O	Long Lane	SU 880760	Cattle	18.76	Pasture	-
21	C	Stroud	SU 903775	Cattle	59.36	Pasture	-
22	O	Elm	SU 413654	Mixed	87.83	Arable, Woodland, Water	Ponds
22	C	Kintbury Holt	SU 397658	Mixed	91.75	Arable, Woodland, Water	Ponds/Streams
23	O	Path Hill	SU 650792	Mixed	91.76	Pasture, Arable	-
23	C	Combe End	SU 650792	Mixed	107.11	Pasture, Arable	-
24	O	North Stoke	SU 609862	Arable	50.81	Arable	-
24	C	North Stoke	SU 619861	Arable	24.14	Arable	-

***O=Organic, C=Conventional; ** Numbers correspond to the figures and tables in the text.**
***** Mixed = crops and livestock**

APPENDIX 2

LIST OF ALL THE MOTH SPECIES CAPTURED ON BOTH ORGANIC AND CONVENTIONAL FARMS.

Abraxas grossulariata
Abrostola tripartita
Acronicta psi
Acronicta rumicis
Agapeta hamana
Agrochola macilenta
Agrotis cinerea
Agrotis clavis
Agrotis exclamationis
Agrotis ipsilon
Agrotis puta puta
Agrotis ripae
Agrotis segetum
Agrotis vestigialis
Alcis repandata repandata
Amphipyra berbera svenssoni
Apamea monoglypha
Apamea oblonga
Apamea remissa
Apamea scolopacina
Apamea sublustris
Apeira syringaria
Aporophyla nigra
Arctia villica britannica
Autographa gamma
Autographa jota
Autographa pulchrina
Brachylomia viminalis
Bupalus piniaria
Cabera exanthemata
Cabera pusaria
Campaea margaritata
Camptogramma bilineata bilineata
Celaena leucostigma
Chilodes maritimus
Chlorochlysta siterata
Chloroclysta truncata
Colostygia pectinataria
Conistra vaccinii
Cosmia pyralina
Cosmia trapezina
Cosmorhoe ocellata
Crambus pratella
Crocallis elinguaria
Cryphia algae
Cydia pomonella
Mesoligia furuncula
Diarsia dahlii
Diarsia rubi
Earias clorana
Eilema complana
Eilema caniola
Eilema depressa
Eilema griseola
Eilema lurideola
Eilema pygmaeola
Elophila nymphaeta
Ennomos quercinaria
Epirrhoe rivata
Erannis defoliaria
Eriocrania subpurpurella
Euphyia unangulata
Eupithecia inturbata
Eupithecia tenuiata
Eurrhypara hortulata
Euthrix potatoria
Euxoa nigricans
Euxoa obelisca grisea
Euxoa tritici
Galleria mellonella
Graphiphora augur
Habrosyne pyritoides
Hadena luteago barrettii
Hemistola chrysoprasaria
Hemithea aestivaria
Hepialus humuli humuli
Hepialus sylvina
Herminia grisealis
Heterogenea asella
Hoplodrina blanda
Hydriomena furcata
Hyloicus pinastri
Hypena proboscidalis
Idaea dimidiata
Idaea straminata
Idaea rusticata atrosignaria
Idaea subsericeata
Ipimorpha subtusa
Lacanobia thalassina
Laspeyria flexula
Ligdia adustata
Mesapamea didyma
Tholera decimalis

Mesopamaea secalis
Micropterix calthella
Miltochrista miniata
Mythimna conigera
Mythimna pallens
Mythimna turca
Mythimna unipuncta
Noctua comes
Noctua janthe
Noctua pronuba
Nola cucullatella
Ochropleura plecta
Odezia atrata
Omphaloscelis lunosa
Opisthograptis luteolata
Orthosia cerasi
Orthosia gothica
Orthosia miniosa
Pechipogo strigilata
Perizoma albulata albulata
Perizoma bifaciata
Phasiphila debiliata
Pheosia tremula
Philbalapteryx virgata
Phologophora meticulosa
Photedes captiuncula expolita
Phragmatobia fuliginosa fuliginosa
Pleuroptya ruralis
Polia nebulosa
Rhyacia simulans
Scoliopteryx libatrix
Scopula emutaria
Scopula imitaria
Scopula rubiginata
Scotopteryx bipunctaria cretata
Scotopteryx chenopodiata
Scotopteryx mucronata umbifera
Spaelotis ravida
Spilosoma lubricipeda
Spilosoma luteum
Thera firmata
Theria primaria
Thethea or or
Timandra comae
Xantharhoe fluctuata
Xanthia aurago
Xestia ashworthii
Xestia c-nigrum
Xestia rhomboidea
Xestia triangulum
Xestia xanthographa

APPENDIX 3

Publications in press resulting from the work in this thesis.

1. Wickramasinghe, L.P., S. Harris, G.Jones & N. Vaughan. Abundance and species richness of nocturnal insects on organic and conventional farms: implications of agricultural intensification for bat foraging. *Conservation Biology*, In press.

2. Jones G., N. Vaughan, D. Russo, L.P. Wickramasinghe & S. Harris. Designing bat activity surveys using time expansion and direct sampling of ultrasound In *Proceedings of the International Bat Echolocation Symposium* (eds Brigham, R.M., Jones, G., Kalko, E., Keeley, B. & Parsons, S.), Austin, Texas, In Press.

Abundance and species richness of nocturnal insects on organic and conventional farms: implications of agricultural intensification for bat foraging

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Abstract: Insects are the principal food for many animals, including bats (Chiroptera), and all species of bat in the United Kingdom feed over agricultural habitats. Bat populations are declining throughout Europe, probably in part due to agricultural intensification. Organic farming prohibits the use of agrochemicals, a major component of agricultural intensification, making it an ideal control for a study of intensive agricultural systems. To evaluate the impact of agricultural intensification on bat foraging, we quantified the availability of their prey by comparing nocturnal aerial insects captured within habitats on 24 matched pairs of organic and conventional farms. Insects were identified to family and moths to species. We compared the abundance of 18 insect families commonly eaten by bats in the United Kingdom between farm type and tested for correlations of abundance with bat activity. Insect abundance, species richness, and moth species diversity were significantly higher on organic farms than on conventional farms. Insect abundance was significantly higher in pastural and water habitats on organic farms than in the same habitats on conventional farms. Of the 18 insect families that are important components of bat diet, five were significantly more abundant on organic farms overall. Some were also more abundant within organic pastural, woodland, and water habitats than on conventional farmland habitats. The activity of bats that mainly ate Lepidoptera was significantly correlated with the abundance of this order. Our observations suggest that

agricultural intensification has a profound impact on nocturnal insect communities. Because bats are resource-limited, a reduction in prey availability through agricultural intensification will adversely affect bat populations. Less intensive farming benefits British bat populations by providing and maintaining diverse and structurally varied habitats, which in turn support a wide selection of insect prey for bats, including insect families that are significant components of the diet of a number of rare bat species.

Introduction

As the major land use in Great Britain (76%), agriculture affects wildlife populations on a national scale (Fuller et al. 1995; Robinson & Sutherland 2002). Agricultural intensification is defined as increased production of agricultural commodities per unit area (Donald et al. 2001) through increased mechanization and use of synthetic chemical fertilizers and pesticides. Intensive farming is multifaceted, and aside from application of agrochemicals for insect removal, other features such as habitat modification also affect insect communities. Organic farming, on the other hand, is a production system in which the use of synthetic fertilizers, pesticides, growth regulators and livestock feed additives are avoided or excluded (Lampkin 1998). These contrasts make the comparison between organic and conventional farming an ideal model system through which to investigate the impact of agricultural intensification on insect communities.

Many populations of insect species have markedly declined over recent years, due primarily to agricultural intensification (Aebischer 1991; Feber et al. 1997; Benton et al. 2002). There have been declines in a number of carabid species in Europe (Luff & Woiwood 1995; Kromp 1999), and studies of long-term trends in invertebrate abundance in Britain showed that most insect groups have declined, notably

Collembola, carabids, and certain predatory insects (Aebischer 1991; Sotherton & Self 2000). However, little is known about the impact of agricultural intensification on the predominantly nocturnal taxa most likely to be eaten by bats.

Many species of bats are declining throughout the United Kingdom and in the rest of Europe (Stebbings 1988; Mitchell-Jones 1995; Hutson et al. 2001). The 16 species of bat in the United Kingdom are protected by European and by national legislation; six of the 16 species have Biodiversity Action Plans (BAPs) assigned to them in accordance with the Convention on Biological Diversity (Anonymous 1995). Agricultural intensification and habitat loss are listed as reasons for the decline of all six United Kingdom BAP species. Not only are the functional ways in which agricultural intensification affects bats not understood, but until recently there have been few data showing that bats are affected by agricultural intensification (Wickramasinghe et al. 2003).

All species of bats in Britain include Lepidoptera as a component of their diet (Vaughan 1997). Ninety percent of Lepidoptera are moths, the majority of which are nocturnal (Janzen 1988; Young 1997). Many species of bats feed predominantly on Lepidoptera (Vaughan 1997). Lepidoptera constitute one of the main dietary components for six species of British bat, three of which (*Rhinolophus ferrumequinum*, *Myotis bechsteinii*, and *Barbastella barbastellus*) have United Kingdom BAPs. As well as featuring heavily in the diet of certain bat species, Lepidoptera play important roles as herbivores and pollinators (Janzen 1987; Barlow & Woiwood 1989). Their host-specificity means they can also act as indicators of plant diversity and land management (Erhardt & Thomas 1991; Luff & Woiwood 1995). Some Trichoptera are also eaten by all the bat species. *Myotis* spp., *Pipistrellus* spp., and *Nyctalus leisleri* are highly reliant on aquatic insects, predominantly dipteran

flies (Swift & Racey 1983; Barlow 1997; Vaughan 1997). In the United Kingdom, Diptera constitute a major part of the diet of 13 species of bat, of which four have BAPs assigned to them. Scaraboid and geotrupid beetles form a major component of the diet for some of the larger species of bat, notably *Rhinolophus ferrumequinum*, *Nyctalus noctula*, and *Eptesicus serotinus* (Hutson et al. 2001).

Many facets of the biology of bats, including low fecundity, longevity, and high survivorship, indicate that they should maintain stable populations close to the carrying capacity of the environment within predictable habitats. These biological traits also suggest that bat communities are limited by resources (Findley 1993).

Bat activity is 61% higher on organic farms, and foraging activity is 84% higher on organic farms than on conventional farms (Wickramasinghe et al. 2003). Therefore, we investigate the functional reasons why bats are less common on conventional farms by studying the effect of agricultural intensification on their prey. We sampled nocturnal and crepuscular aerial insects on 24 matched pairs of organic and conventional farms. Our research goals were to (1) examine the relationship between the abundance and species richness of insects and organic and conventional farm type, (2) assess the impact of agricultural intensification on specific insect families important in the diet of bats (referred to as "key" insect families), and (3) investigate the relationship between bat activity and insect abundance.

Methods

Study sites and sampling protocol

The study was carried out in 2000 and 2002 from April to September in southern England and Wales. We used matched pairs of organic and conventional farms to standardize various characters within a pair as much as possible. The exception was

farm type (organic and conventional). This kind of paired design has been used widely in previous studies (Feber et al. 1997; Letourneau & Goldstein 2001). The organic farms were certified by the official national certifying bodies in the United Kingdom (The Soil Association Certification Limited, Bristol, and Organic Farmers and Growers Ltd, Shrewsbury, Shropshire). Twenty-four farm pairs were matched. Each certified organic farm was paired with a conventional farm no more than 5 km away, thereby controlling for geographic variation. Due to the absence of a national list of conventional farms, we selected these farms by asking organic farmers about the nearest conventional farm with a similar business that would be suitable for study. The business types of the 24 pairs of farms sampled consisted of 54% livestock, 41% mixed (crops and livestock), and 4% crops only. Two pairs were located in Wales and the remaining 22 pairs in southern England. We conducted habitat surveys (Anonymous 1990) on all sites and entered data into a geographic information system (GIS) application (ArcView version 3.2 and ArcView Spatial Analyst, Environmental Systems Research Inst.). Habitat areas were calculated by using this software. The sizes of the farms within a pair were kept as similar as possible and insects were sampled within one or more of four habitats. Habitats were pastural, arable, water, and woodland, with the order of habitats sampled within a pair kept the same. We measured hedgerow height with a 1-m ruler (accuracy ±1 cm).

Because we sampled bats and insects simultaneously, temporal differences were controlled for by sampling on consecutive nights and by following a strict sampling protocol. This protocol ensured that environmental variables were controlled for between nights. The temperature measured at dusk had to be within 4 °C of that of the previous night for sampling to commence on the second night. Insects become less active below 10 °C and prolonged rain would damage sensitive field equipment, so

sampling was abandoned if the temperature dropped below 10 °C or if heavy rain set in (Rydell et al. 1996). If sampling was abandoned half way through a pair, the second farm was sampled on the next night. If this night was also unsuitable the whole pair was resampled. This meant there was a gap of no more than one night between sampling of farms in a pair. Methods for determining bat activity (number of bat passes recorded by direct sampling of ultrasound) and acoustic identification are described in Wickramasinghe et al. (2003).

Insect capture methods

Because all insect capture methods are biased toward catching prey of a certain size, mass, or flight behavior (Muirhead-Thompson 1991; Sutherland 1998), we used a combination of portable Heath light traps, flight intercept traps, and sweep netting. The Heath trap has a blacklight bulb as the attractant, and is powered by a 12-volt motorcycle battery. We made 100 sweeps at three randomly chosen sampling points no less than 15 m apart. A total of 300 sweeps with a sweep net were made in a figure of eight within each habitat type. The flight intercept trap consisted of a fine black net (53 x 97 cm) stretched between two poles and placed at a 90° angle to the direction of the wind. Fast-flying insects hit the netting and fell into a tray (35 x 54cm) containing a weak solution of detergent. One of each type of trap was placed near a hedgerow within each habitat sampled, each trap > 15 m from the next. On most occasions the traps could be placed in different fields of the same habitat.

The timing of sampling was important because bat activity was also being sampled. To standardize sample collection, we activated insect traps at dusk in each of the selected habitats, and collected the catch when the night's bat sampling ended (typically after 5 hours). The timing of insect sampling coincided with peak foraging

activity of bats and ended before insect abundance dropped (Racey & Swift 1985). At the end of the sampling night, we sealed the light traps and transported the trap catch to the laboratory. We preserved the flight intercept trap catch in 70% alcohol for later identification. A cotton pad soaked with ethyl acetate was dropped into the light traps, which were then resealed and left for 9 hours, after which the insects were transferred into sample bottles. We stored moths and delicate flies separately to prevent scale loss and damage to wing membranes. The sweep net catches were stored in sample bottles. Insect catches were then frozen for identification at a later date.

Insect identification

Insects were identified to family and the moths were identified to species (Colyer & Hammond 1968; Unwin 1981; Harde 1984; Sterry 1997; Skinner 1998). If identification was not possible due to missing parts or if there was uncertainty, the insects were classified as "not identifiable" and left out of the analysis of diversity. Eighteen insect families that are important components of bats' diets (Vaughan 1997) were chosen for study. Each key insect family is a major component of the diet of at least one bat species in the United Kingdom (Table 1).

Bats were grouped by feeding trait or as one of the six species with a BAP. Insect families were grouped by order to investigate the relationship between the activity of the bat groups, determined by the number of bat passes, (Wickramasinghe et al. 2003), and the abundance of their main food groups. We also investigated relationships between prey-type specialists and key insect families. The combined abundance of Lepidoptera and Diptera were also tested for relationships with all the bat groups because many bat species take both orders of insect as part of their diet.

After counting and identification, the dry masses of total trap catches within habitats on organic and conventional farms were recorded. The total catch for each habitat on each farm was dried in an oven at 50 °C, for up to 40 hours. The mass was measured at intervals until constant, after which final measurements were taken.

The use of higher taxa, such as family richness, as a surrogate for species richness has been validated in a number of studies (Balmford et al. 1996*a*, *b*; Hughes et al. 2000). We explored the relationship between species richness and family richness for moths.

Statistical methods

We analysed the differences between farm types with paired-sample *t* tests; data were log transformed ($\log_{10}(X+1)$) if necessary to achieve normality in the differences (Zar 1999). These statistical analyses were performed using Minitab version 13 (Ryan & Joiner 1994). To evaluate the predictive power of using family as an indicator for species richness we used linear regression models on the log transformed data ($\log_{10}(X+1)$) for both farm types separately. We calculated Shannon-Wiener diversity indices to test for differences in diversity between farm type. The differences between farm types in the number of insects belonging to key insect families and in species richness were analysed using the Wilcoxon paired-sample test, because the data were not normally distributed (Zar 1999).

Results

We captured 7598 insects in approximately 240 hours of sampling; 7548 were identified to family (Table 2) and 1189 of 1239 moths were identified to species

(Appendix 1). Some of the moth species captured were agricultural pests (e.g. turnip moth *Agrotis segetum* and codling moth *Cydia pomonella*).

Organic and conventional farms did not differ statistically for mean temperature, mean wind speed, farm area, or areas of habitats sampled (Wickramasinghe et al. 2003). Hedgerow height was significantly greater on organic farms than on conventional farms (Wickramasinghe et al. 2003). There was no correlation between number of insects and hedgerow height or wind speed, but a marginally significant correlation was found between insect abundance and temperature (Spearman's coefficient correlation r_s=0.286, df=46, p=0.049).

Insect abundance

Total insect abundance was significantly different between farm type; more insects were found on organic than on conventional farms (t=6.55, df=23, p=0.000; Fig. 1a). When analysed by habitat type, insect abundance was significantly higher on organic pastural (t=4.35, df=21, p=0.000) and water habitats (t=3.03, df=8, p=0.019) than on the same habitats on conventional farms. No statistical difference between farm type was evident in arable (t=1.54, df=8, p=0.167) or woodland habitats (t=1.85, df=10, p=0.097).

Insect dry mass was significantly higher on organic farms than on conventional farms (t=2.11, df=23, p= 0.046; Fig. 1b). Within individual habitats, insect dry mass was significantly higher in pastural (t=3.97, df=20, p= 0.001) and woodland habitats (t=2.94, df=9, p= 0.017) than on the same habitats on conventional farms.

Insect richness and diversity

There was a significant correlation between moth species richness and moth family

richness for both farm types and the predictive power of both models was high (linear regressions: organic farms: F=107.3, df=1, 22, r^2=0.83, p=0.000; conventional farms: F=61.9, df=1, 22, r^2=0.74, p=0.000). Therefore, we used family richness as an indicator of species richness, as advocated by Balmford et al. 1996*a*, *b*. The difference in the total number of insect families between farm type was significant, with a higher family richness (indicating higher species richness) on organic than on conventional farms (Wilcoxon paired-sample test Z=-3.045, df=23, p=0.002; Fig. 1c). Moth species richness was also significantly higher on organic than on conventional farms (Wilcoxon paired-sample test Z=-3.360, d.f.=23, P=0.001), as was moth species diversity (Wilcoxon paired-sample test Z=-2.277, d.f.=23, P=0.023).

Key insect family abundance

Of the 18 key insect families, five were captured in significantly higher numbers on organic than on conventional farms (Table 2). These were two lepidopteran families, two dipteran families, and a coleopteran family. There were also significant differences in the abundance of the key families in different habitats between farm types (Table 3). Over water habitats the abundance of Psychodidae was significantly higher on organic farms than on conventional farms. In woodland habitats the abundance of the dipteran family Chironomidae was significantly higher on organic farms. Over pastural habitats, Carabidae, Scarabaeidae, Chironomidae, Psychodidae, Arctiidae, Noctuidae, and Geometridae were all captured in significantly higher numbers over organic farms than over conventional farms. There was no significant difference in key groups between farm type within arable habitats. No key insect families were significantly more abundant on conventional farms than on organic farms.

Relationship between key insect family differences and bat activity

Certain *Myotis* bat species and the dipteran families they eat are both significantly more abundant on organic farms overall and within organic water habitats than on conventional farmland habitats. Specifically, there was significantly more *M. daubentonii* and *M. bechsteinii* activity on organic farms, and significantly more *M. bechsteinii* and *M. brandtii* activity over organic water habitats (Wickramasinghe et al. 2003). This is consistent with Psychodidae and Chironomidae being more abundant on organic farms, with Psychodidae being more abundant on organic pasture and water habitats, and with Chironomidae being more abundant on organic pasture and woodland habitats.

There was no relationship between total bat activity and total insect abundance (Spearman's coefficient correlation, r_s=0.175, df=46, p=0.235). The activity of bats that mainly eat Lepidoptera (as defined in Table 1) and the abundance of Lepidoptera were significantly correlated (r_s=0.239, df=46, p=0.044). The activity of this group of bats was also significantly correlated with the abundance of Lepidoptera and Diptera combined (r_s=0.346, df=46, p=0.016).

Discussion

Changes in insect abundance

The paired experimental design we used standardized for as many variables as possible between farms in a pair with the exception of farm type, the main difference being the presence or absence of agrochemicals.

In this study, insect family richness was used as an indicator of species richness. Balmford et al. (1996*a*, *b*) state that even when relationships between higher

taxa and numbers of species are significant, they vary in strength and the prediction of absolute species richness may be low. The use of higher taxa in this study was validated by the high predictive power resulting from the linear regression models for both farm types. However, variation in predictive power may not be important if the goal of higher taxa surveys is to rank the relative richness of sites (Balmford et al. 1996*a*, *b*).

Nocturnal and crepuscular aerial insect abundance and species richness was significantly higher on organic farms than on conventional farms. The primary reason for this difference is likely to be the use of agrochemicals on conventional farms. Pesticides reduce insect numbers of both target and nontarget species, even in unsprayed headlands through spray drift (Chiverton & Sotherton 1991; Sotherton 1991; de Snoo 1999). In the United Kingdom antihelminthic drugs such as avermectin, used for cattle and sheep, may reduce insect fauna in dung (Strong 1992), especially scarabiod and geotrupid dung beetles, both of which are an important part of the diet of *R. ferrumequinum*, *N. noctula*, and *E. serotinus* (Hutson et al. 2001). Other researchers have found that insect diversity is generally lower in more intensively managed fields (di Giulio et al. 2001; Benton et al. 2002). In addition to direct insecticidal effects, herbicides used in intensively managed farms have indirect effects on invertebrate populations because they kill their food plants (Moreby & Southway 1999; Robinson & Sutherland 2002). Insect species richness is positively related to plant species richness and plant functional group richness (Strong et al. 1984; Haddad et al. 2001). Because organic standards prohibit the use of herbicides, the condition of habitats on organic farms is expected to be better in terms of habitat structure and plant diversity than that of habitats on conventional farms.

The concept of landscapes as complex mosaics of habitats varying in quality with respect to different groups of organisms has been the subject of a number of recent studies (Wiens 1995; Gascon et al. 1999; Ricketts et al. 2001). Patches of habitat with varying quality are likely to underlie the differences we found in insect abundance between the different farm types. Synthetic chemicals and nutrients such as nitrogen and phosphorous are major contributors to aquatic pollution (Angier et al. 2002). Excess phosphorous in a system primarily affects aquatic life, and excess nitrogen affects aquatic life and can be damaging to human health (Hapeman 2002). An increase in organic pollution from manure runoff results in a gradual decrease in the oxygen content of the water, which can affect sensitive organisms such as trichopteran larvae and Plecoptera, although the actual impact depends on the severity of oxygen depletion (Mason 2002). As the oxygen content increases at a site, the numbers of chironomids and other insects may also increase (Mason 2002), which may explain the higher activity of such insects on organic farms where organic runoff is likely to occur. Organic water habitats had the highest number of insects trapped of all the habitats sampled, followed by pastural habitats. Hundreds of Diptera were occasionally caught within one sampling period on organic farms over water habitats; this explains the large differences in abundance observed in certain farm pairs (6, 12 and 14) of Fig. 1a. The significantly higher insect abundance over organic water habitats was probably due to good water quality (i.e. the absence of synthetic chemical runoff), and the fact that trees or bushes surrounded almost all these sites. The presence of trees and bushes provide shelter for emerging aquatic insects and dead leaf beds which are important habitats for other insect groups.

Although the main difference between organic and conventional farms is the use of synthetic chemicals, there are undoubtedly mechanisms other than agrochemical

use linking intensive farming with the reduction of insects. Kirby (2001) identified habitat continuity and structural variation as the two most important factors in maintaining insect populations at a site. Vegetation structure at the microhabitat level is also important for insect communities, and a reduction in grazing intensity, for example, enhances insect diversity (Kruess & Tscharntke 2002). Insect densities are generally higher nearer vertical landscape elements than in open areas (Lewis 1970; Lewis & Dibley 1970).

Implications for bat foraging

As well as the clear difference in total insect number between farm type, there was also a greater abundance of insects belonging to five key insect families on organic farms. These included lepidopteran, coleopteran, and dipteran families.

The high numbers of larger insects such as Lepidoptera and Coleoptera on organic farms explains the higher dry mass measurements on this farm type both overall and within pastural habitats. A study comparing trends in moth numbers in different habitats showed a general decline in farmland populations but little change in woodland populations (Woiwood & Harrington 1994). Our results support the hypothesis that agricultural intensification has contributed to this decline.

Our comparison of the activity of bat species most affected by agricultural intensification and the abundance of those insect families most commonly eaten by these bats, showed that a number of key insect families were significantly more abundant on organic farms. This was associated with higher activity levels of the bat species that preyed on those key insect families. Although total bat activity was not significantly correlated with total insect abundance, the activity of bats whose diet consisted mainly of Lepidoptera was significantly correlated with the abundance of

Lepidoptera and with the abundance of Lepidoptera and Diptera combined. Our results suggest that increasing the numbers of individuals in key families of insects will increase the numbers of their bat predators.

Conclusions

Agricultural intensification had a profound impact on nocturnal and crepuscular aerial insect abundance, and certain insect families, many of which are host-specific, were less common on conventional farms than on organic farms. In particular insect families important in bat diet were adversely affected by agricultural intensification. Changes in land use through agricultural intensification have reduced resource abundance for bats and reduced the stability and predictability of such food resources. Because bat communities are resource-limited (Bonaccorso 1979; Findley 1993), our data support the hypothesis that agricultural intensification has been a factor in the reduction in the numbers of key dietary components for bats and that this reduction has led to reduced bat activity on conventional farms. Significant correlations between the activity of bats and the abundance of their prey support assumptions in the United Kingdom BAPs that agricultural intensification has been a significant factor leading to bat population declines. Furthermore, our data suggest that managing farms to maximise insect abundance, especially that of key insect families, by maintaining diverse and structurally varied habitats and reducing agrochemical use, would benefit bat populations.

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Table 1. Key insect families important in bat diets in Britain (families that make up over 10% of diet).

Insect order	Family	BAP species[a]						Other species[b]
		R.f	*R.h*	*M.b*	*B.b*	*P.p*	*P.py*	
Coleoptera	Carabidae	✓						*N.n, E.s*
	Scarabaeidae	✓						*N.n, N.l, E.s*
Diptera	Tipulidae	✓	✓			✓	✓	*M.br, M.m, M.n, M.d, N.l, N.n, P.a, P.au*
	Culicidae	✓	✓					*N.l, N.n*
	Anisopodidae	✓	✓					*M.m, M.br, N.n, P.a*
	Sciaridae							*M.n, N.l*
	Chironomidae	✓				✓	✓	*M.br, M.d, P.n, N.l, N.n, P.a*
	Dolichopoidae							*M.n, N.l*
	Ceratopogonidae		✓			✓	✓	*N.l*
	Psychodidae					✓	✓	*M.m*
Lepidoptera	Pyralidae							*N.l, P.a*
	Arctiidae				✓			*P.a*
	Noctuidae	✓		✓				*N.l, P.a, P.au*
	Geometridae	✓						*N.l, P.a, P.au*
Trichoptera	Limnephilidae	✓				✓		*M.d*
	Brachycentridae	✓	✓			✓	✓	*M.d*
	Molannidae	✓	✓			✓	✓	*M.d*
	Beraeidae	✓	✓			✓	✓	*M.d*

[a]Bat species that have biodiversity action plans (BAPs) in the United Kingdom.

[b]*Rhinolophus ferrumequinum (R.f), R. hipposideros (R.h), M. bechsteinii (M.b), M. nattereri (M.n), M. mystacinus (M.m), M. brandtii (M.br), Myotis daubentonii (M.d).*

Barbastella barbastellus (B.b), Pipistrellus pipistrellus (P.p), P.pygmaeus (P.py), P. nathusii (P.n), N. leisleri (N.l), Nyctalus noctula (N.n), Eptesicus serotinus (E.s), Plecotus auritus (P.au), Plecotus austriacus (P.a).

Table 2. List of insect families identified with mean numbers of insects captured in each farm type.

Family[a]	Organic farm Mean ±SEM[b]	Conventional farm Mean ±SEM[b]	Family[a]	Organic farm Mean ±SEM[b]	Conventional farm Mean ±SEM[b]
Forficulidae	0.42±0.16	0.17±0.10	Tipulidae[c]	3.50±5.20	2.35±0.94
Berytidae	0.21±0.08	0.04±0.04	Trichoceridae	3.02±0.66	1.82±0.21
Anthocoridae	1.03±0.49	1.25±0.26	Culicidae[c]	1.67±1.20	0.44±0.24
Corixidae	11.41±6.13	4.15±0.44	Anisopodidae[c]	3.95±0.95	6.04±1.97
Cercopidae	1.13±0.28	0.33±0.13	Scatopsidae	1.62±0.30	5.02±2.08
Cicadellidae	0.25±0.10	0.29±0.11	Cecidomyiidae	15.63±3.63	8.32±2.21
Aphididae	0.04±0.04	0.17±0.08	Sciaridae[c]	6.13±1.94	8.50±5.38
Carabidae[c]	1.04±0.30 *	0.29±0.42	Chironomidae[c]	67.17±15.55 **	33.16±13.09
Hydrophilidae	1.65±0.69	1.22±0.16	Blephariceridae	0.25±0.11	0.04±0.04
Byrrhidae	0.29±0.11	0	Phoridae	1.79±0.68	0.17±0.10
Scarabaeidae[c]	1.65±0.61	0.35±0.17	Lonchopteridae	3.86±1.05	1.06±0.19
Chrysomelidae	2.31±0.70	0.87±0.18	Dolichopodidae[c]	0.83±0.29	0.63±0.22
Hydraenidae	2.23±1.28	1.45±0.63	Certatopogonidae[c]	1.69±0.10	2.68±2.37
Curculionidae	1.04±0.33	1.04±0.23	Psychodidae[c]	13.15±3.08 **	5.86±1.69

Syrphidae	0.04±0.04	0.17±0.08	Limnephilidae[c]	2.44±1.39	1.58±0.60
Lauxaniidae	0.17±0.08	0.13±0.07	Brachycentridae[c]	0.29±0.19	0.04±0.04
Sepsidae	2.45±0.61	3.15±0.98	Hydropsychidae	0.29±0.14	0
Opomyzidae	0.42±0.13	1.04±0.27	Polycentropidae	0.42±0.20	0
Scathophagidae	1.93±0.82	1.22±0.27	Psychomyiidae	0.71±0.22	0
Muscidae	0.46±0.18	0.13±0.09	Odontoceridae	1.20±0.33	0.13±0.07
Mycetophilidae	0.46±0.33	0.33±0.12	Glossomatidae	2.43±0.65	2.11±0.49
Ptychopteridae	0.17±0.10	0.13±0.07	Molannidae[c]	1.00±0.56	0.33±0.22
Cochylidae	0.33±0.12	0.29±0.09	Beraeidae[c]	0.75±0.30	0.61±0.20
Pyralidae[c]	1.96±0.92	2.33±0.56	Cynipidae	0.29±0.18	0.04±0.04
Micropterigidae	3.96±1.06	2.12±0.42	Ichneumonidae	1.85±0.46	1.72±0.41
Eriocraniidae	0.20±0.10	0.17±0.08	Formicidae	0.38±0.12	0.38±0.13
Yponomeutidae	0.88±0.28	0.58±0.16			
Arctiidae[c]	2.36±1.25	1.22±0.92			
Noctuidae[c]	17.05±4.68 **	8.35±2.10			
Lasiocampidae	0.17±0.10	0.17±0.10			
Geometridae[c]	4.04±1.12 **	2.15±0.81			

1 [a]Only families with total numbers of five and above are shown.

[b]SEM=standard error of the mean.

[c]Key insect families important to the diet of bats in the United Kingdom. Probability: *$p<0.05$, **$p<0.01$. Degrees of freedom = 23.

Table 3. Mean ± standard error of the mean for numbers of insects captured in each habitat and statistical difference in abundance of key insect families between farm pairs.

Family[a]	Pasture:		Arable:		Woodland:		Water:	
	Organic	Conventional	Organic	Conventional	Organic	Conventional	Organic	Conventional
Carabidae	0.76±0.25 *	0.23±0.11	0.43±0.43	0	0.18±0.18	0.09±0.09	0.57±0.30	0.14±0.14
Scarabaeidae	0.43±0.18 *	0.05±0.05	2.71±1.49	0	0.18±0.12	0.27±0.14	1.43±0.57	0.57±0.37
Tipulidae	1.86±0.72	0.86±0.34	0.29±0.18	0.71±0.42	3.73±1.96	1.82±0.76	0.29±0.29	0.43±0.20
Culicidae	1.76±1.37	0.38±0.25	0.14±0.14	0	0.18±0.12	0.18±0.12	0	0
Anisopodidae	2.09±0.54	2.28±0.58	1.14±0.63	6.14±4.71	3.18±1.12	3.91±2.19	1.14±0.63	1.57±0.97
Sciaridae	3.52±1.09	4.95±1.56	2.57±1.23	5.29±4.03	0.63±0.54	4.45±6.19	6.86±5.78	2.00±0.95
Chironomidae	36.24±8.59 **	15.33±3.43	11.71±2.57	46.85±36.94	18.00±6.18 *	4.27±1.51	81.57±32.34	14.14±3.19
Dolichopodidae	0.48±0.19	0.24±0.11	0	0.14±0.14	0.55±0.39	0.55±0.31	0.57±0.30	0.14±0.14
Ceratopogonidae	1.47±1.06	0.33±0.23	0.29±0.29	8.14±8.14	0.45±0.37	0	0.43±0.43	0
Psychodidae	5.86±1.66 *	3.57±1.18	3.00±1.48	2.57±1.23	5.18±3.08	1.81±0.74	16.14±7.15 *	4.00±3.00
Pyralidae	1.14±0.58	1.52±0.54	1.43±1.43	1.71±0.94	0.36±0.20	0.45±0.21	0.57±0.43	1.00±0.58
Arctiidae	1.28±0.55 *	0.10±0.10	1.57±1.06	1.43±0.97	1.55±1.45	1.36±1.26	0.29±0.18	0.29±0.18

Noctuidae	10.71±3.01 **	4.61±1.00	12.43±5.31	4.43±2.02	6.09±1.01	4.81±1.74	4.29±2.54	2.71±1.22
Geometridae	2.24±0.54 **	0.76±0.28	1.86±0.96	2.14±0.91	2.27±1.20	1.45±0.80	1.71±0.36	0.71±0.57
Limnephilidae	0.42±0.20	0.47±0.18	0.43±0.43	1.00±0.58	3.09±2.71	0.64±0.36	1.71±1.23	2.00±1.00
Brachycentridae	0.33±0.21	0.05±0.05	0	0	0	0	0	0
Molannidae	0.53±0.53	0.05±0.05	0.14±0.14	0	0.72±0.55	0.45±0.45	0.57±0.57	0.23±0.23
Beraeidae	0.52±0.43	0	0.57±0.57	0.43±0.30	0	0	0.43±0.30	1.57±0.90
Unidentified	5	2	8	4	2	5	1	5

[a]Key insect families are families important in the diets of British bats. Probability: *p<0.05, **p<0.01. A significant result indicates higher abundance on organic habitats. In no family was there a significantly higher insect abundance on conventional habitats. Degrees of freedom: pasture 20, woodland 9, water 7, arable 7 (Wilcoxon paired-sample test).

Figure legend

Figure 1(a) Differences in the numbers of insects per farm pair (organic minus conventional data). (b) Differences in total insect dry mass per farm pair (organic minus conventional data). (c) Differences in insect family richness per farm pair (organic minus conventional data). Black bars indicate more insects, higher dry mass or higher family richness on organic farms, white bars more insects, higher dry mass or higher family richness on conventional farms (n=24 pairs).

Appendix 1.

List of all the moth species captured on both organic and conventional farms.

Abraxas grossulariata	*Diarsia dahlii*
Abrostola tripartita	*Diarsia rubi*
Acronicta psi	*Earias clorana*
Acronicta rumicis	*Eilema complana*
Agapeta hamana	*Eilema caniola*
Agrochola macilenta	*Eilema depressa*
Agrotis cinerea	*Eilema griseola*
Agrotis clavis	*Eilema lurideola*
Agrotis exclamationis	*Eilema pygmaeola*
Agrotis ipsilon	*Elophila nymphaeta*
Agrotis puta puta	*Ennomos quercinaria*
Agrotis ripae	*Epirrhoe rivata*
Agrotis segetum	*Erannis defoliaria*
Agrotis vestigialis	*Eriocrania subpurpurella*
Alcis repandata repandata	*Euphyia unangulata*
Amphipyra berbera svenssoni	*Eupithecia inturbata*
Apamea monoglypha	*Eupithecia tenuiata*
Apamea oblonga	*Eurrhypara hortulata*
Apamea remissa	*Euthrix potatoria*
Apamea scolopacina	*Euxoa nigricans*
Apamea sublustris	*Euxoa obelisca grisea*
Apeira syringaria	*Euxoa tritici*
Aporophyla nigra	*Galleria mellonella*
Arctia villica britannica	*Graphiphora augur*
Autographa gamma	*Habrosyne pyritoides*
Autographa jota	*Hadena luteago barrettii*
Autographa pulchrina	*Hemistola chrysoprasaria*
Brachylomia viminalis	*Hemithea aestivaria*
Bupalus piniaria	*Hepialus humuli humuli*
Cabera exanthemata	*Hepialus sylvina*
Cabera pusaria	*Herminia grisealis*
Campaea margaritata	*Heterogenea asella*
Camptogramma bilineata bilineata	*Hoplodrina blanda*
Celaena leucostigma	*Hydriomena furcata*
Chilodes maritimus	*Hyloicus pinastri*
Chlorochlysta siterata	*Hypena proboscidalis*
Chloroclysta truncata	*Idaea aversata*
Colostygia pectinataria	*Idaea dimidiata*
Conistra vaccinii	*Idaea straminata*
Cosmia pyralina	*Idaea rusticata atrosignaria*
Cosmia trapezina	*Idaea subsericeata*
Cosmorhoe ocellata	*Ipimorpha subtusa*
Crambus pratella	*Lacanobia thalassina*
Crocallis elinguaria	*Laspeyria flexula*
Cryphia algae	*Ligdia adustata*
Cydia pomonella	*Mesapamea didyma*
Mesoligia furuncula	*Tholera decimalis*

Mesopamaea secalis
Micropterix calthella
Miltochrista miniata
Mythimna conigera
Mythimna pallens
Mythimna turca
Mythimna unipuncta
Noctua comes
Noctua janthe
Noctua pronuba
Nola cucullatella
Ochropleura plecta
Odezia atrata
Omphaloscelis lunosa
Opisthograptis luteolata
Orthosia cerasi
Orthosia gothica
Orthosia miniosa
Pechipogo strigilata
Perizoma albulata albulata
Perizoma bifaciata
Phasiphila debiliata
Pheosia tremula
Philbalapteryx virgata
Phologophora meticulosa
Photedes captiuncula expolita
Phragmatobia fuliginosa fuliginosa
Pleuroptya ruralis
Polia nebulosa
Rhyacia simulans
Scoliopteryx libatrix
Scopula emutaria
Scopula imitaria
Scopula rubiginata
Scotopteryx bipunctaria cretata
Scotopteryx chenopodiata
Scotopteryx mucronata umbifera
Spaelotis ravida
Spilosoma lubricipeda
Spilosoma luteum
Thera firmata
Theria primaria
Thethea or or
Timandra comae
Xantharhoe fluctuata
Xanthia aurago
Xestia ashworthii
Xestia c-nigrum
Xestia rhomboidea
Xestia triangulum
Xestia xanthographa

1
2

Figure 1(a)

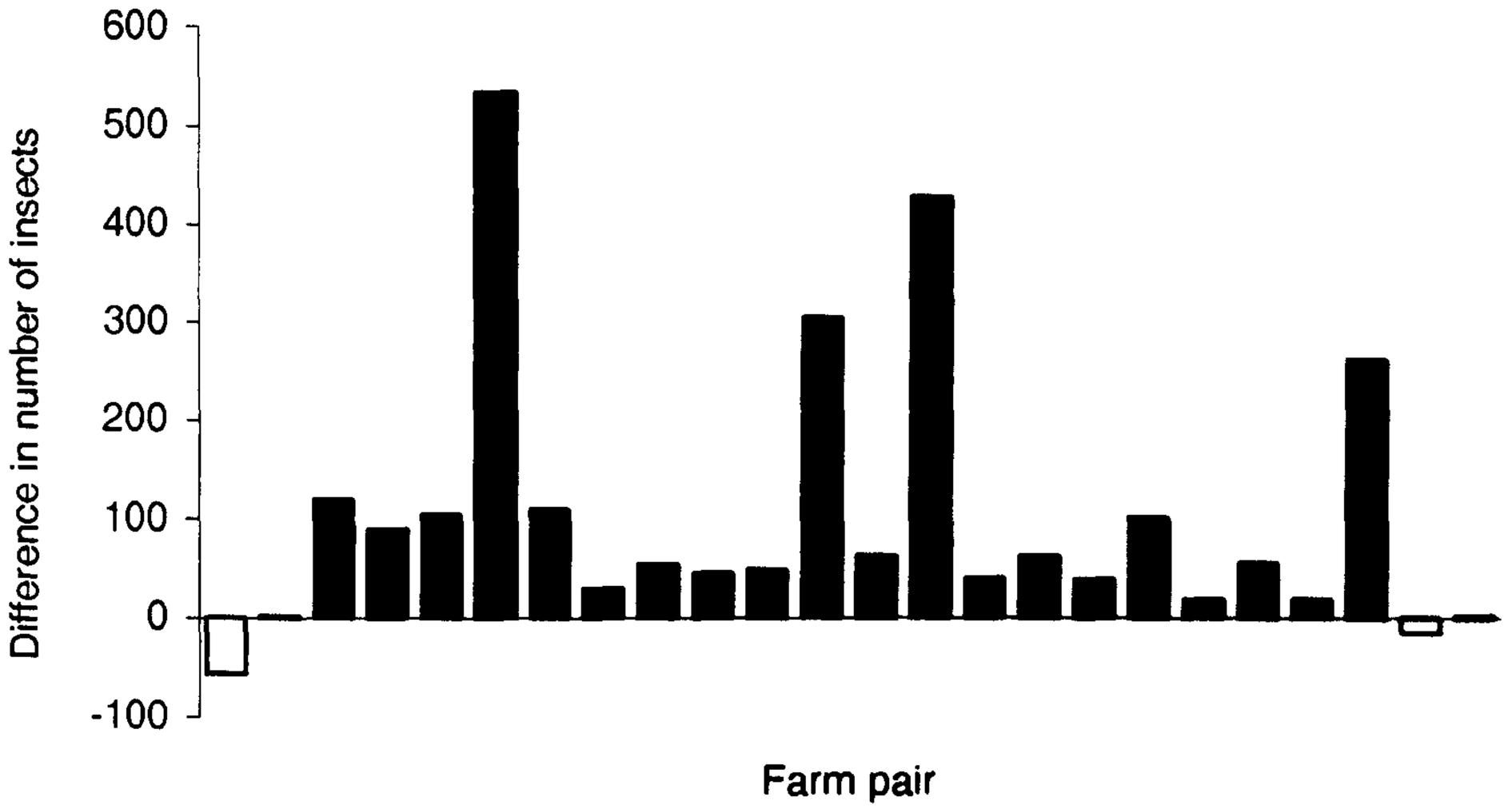


Figure 1(b)

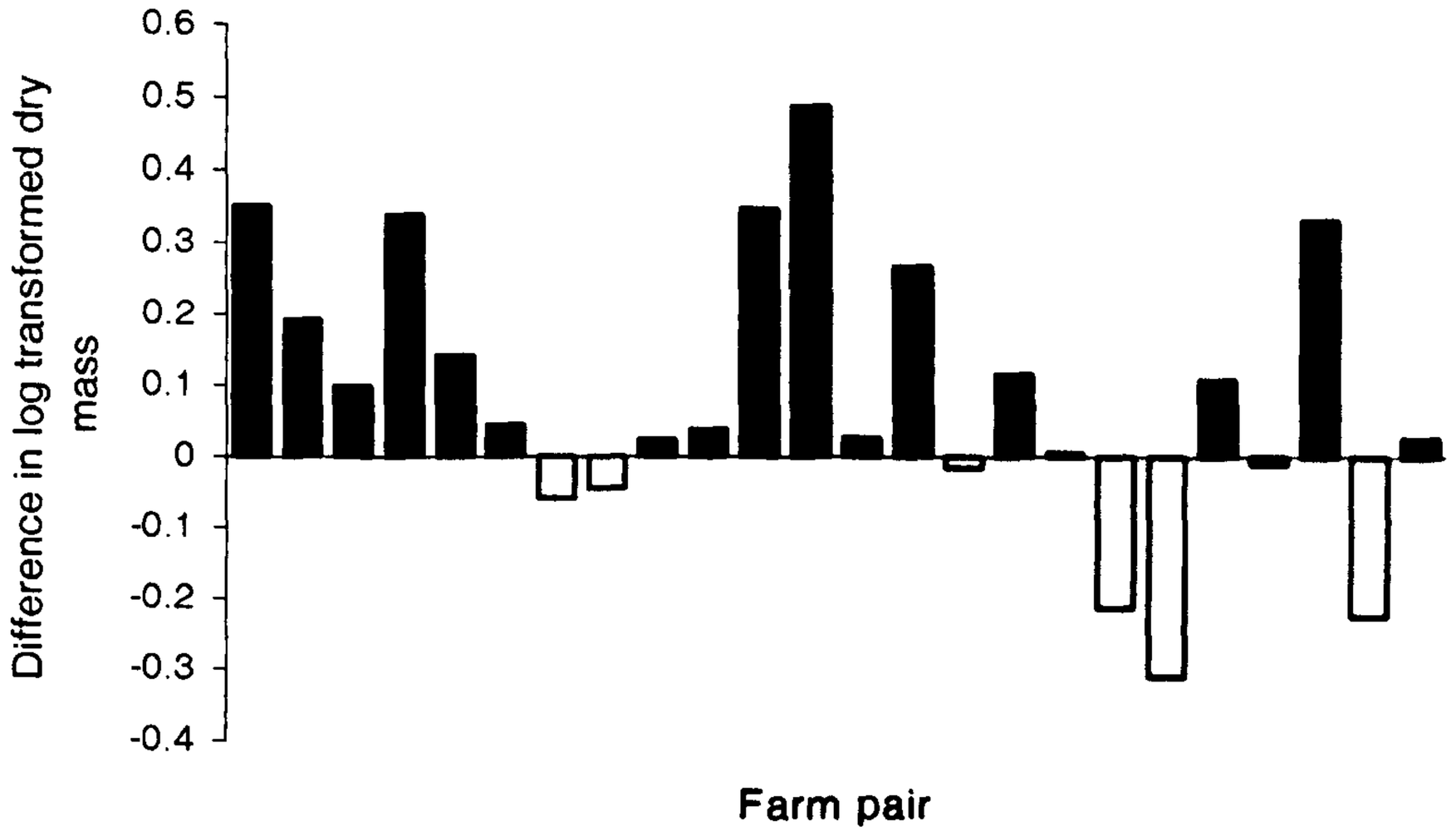
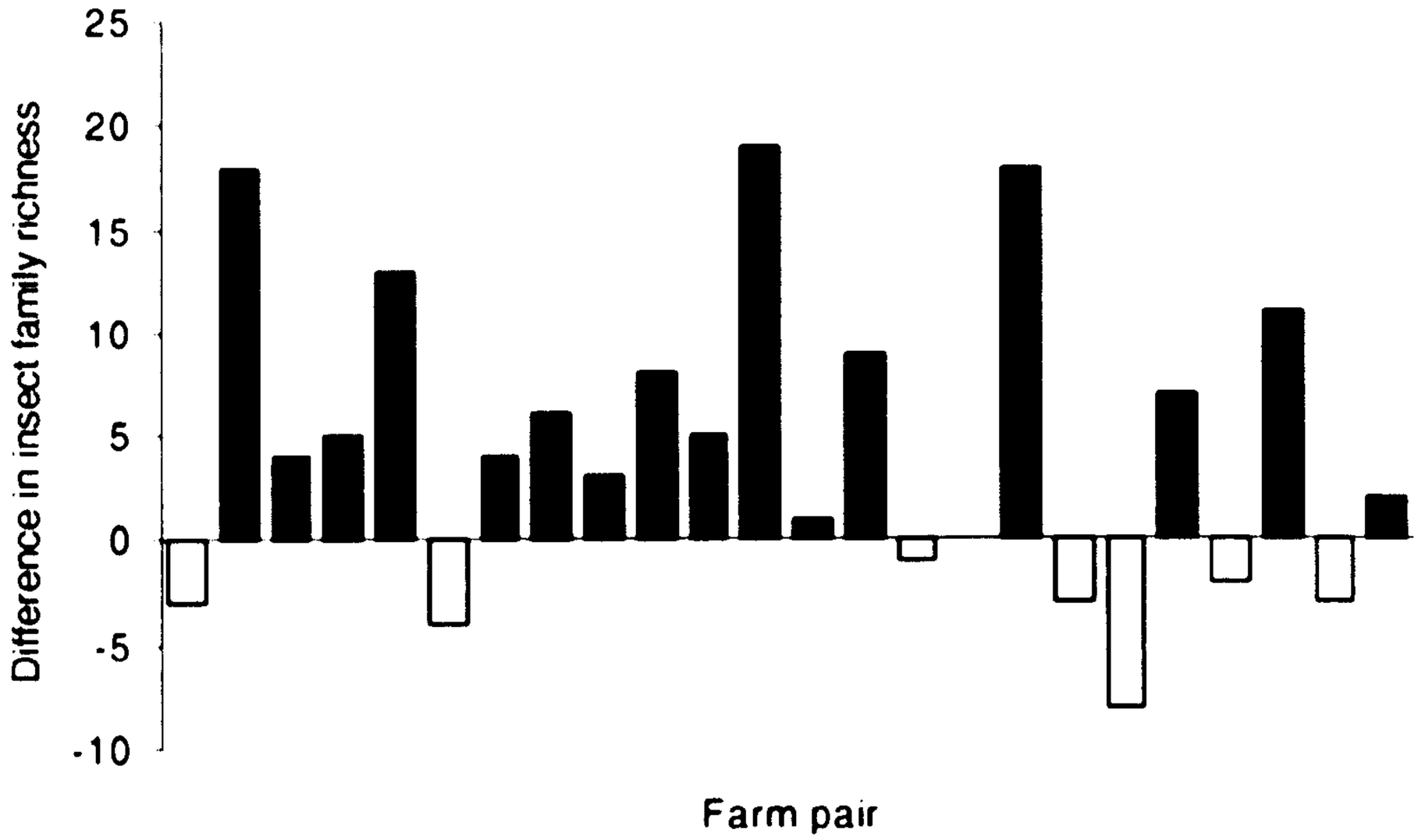


Figure 1(c)


25
20
15
10
5
0
-5
-10
Difference in insect family richness
Farm pair

DESIGNING BAT ACTIVITY SURVEYS USING TIME EXPANSION AND DIRECT SAMPLING OF ULTRASOUND

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We reviewed acoustic studies that use time expansion methods to determine habitat use by bats in Europe. Species identification can be quantified by using discriminant function analysis or neural networks. These methods maximize the information recorded from echolocation calls, and allow confident classification of calls to species. Because the recording equipment is expensive, surveys typically involve one recording device and mobile sampling along transects. We walk transects for a fixed time starting at a fixed time after sunset. Bats are detected by listening on frequency division mode, and calls are time-expanded on detection. Our methods involve sampling replicates of each habitat, and visiting habitats in random order

over the summer. We estimate foraging success by calculating the ratio of feeding buzzes to passes. We illustrate our methods by describing habitat surveys in Britain and southern Italy. The speciose bat community in Italy presents considerable challenges for acoustic identification, but nevertheless we achieved a high rate of correct classification of calls to species. More recently, we have used paired sampling of organic vs. conventional farms together with direct sampling of ultrasound to determine whether intensive farm management has a detrimental effect on bat activity. Direct sampling overcomes the wasted download time inherent in time expansion, and allows acquisition of extended high quality recordings.

Key words: *acoustic identification, bat activity, bat detectors, habitat use, ultrasound*

Being nocturnal, bats are difficult animals to survey visually. Because many species have distinctive echolocation and social calls there has been increasing interest on developing acoustic surveys of their activity (e.g., Kalkounis et al. 1999; Seidman and Zabel 2001; Vaughan et al. 1997a). Survey methods should rely on a robust method for acoustic

identification. Although some authors have taken a qualitative approach to acoustic identification of bat species (O'Farrell et al. 1999), we agree with Barclay (1999) and argue that acoustic surveys must be quantitative and objective. Objectivity is especially important to control for differences in identification abilities among recorders, and if surveys are to be repeated in the future, for example to assess long-term changes in bat activity. Here we describe some methods and results from studies of bat activity using time expansion detectors. Important assumptions relating to studies of bat activity by acoustic monitoring (e.g., relating activity to habitat quality, whether feeding buzzes accurately reflect foraging activity) are reviewed by Hayes (2000). These assumptions relate to all detector methods.

Recording: Time Expansion and Direct Sampling

Several types of detector have been used for acoustic surveys. Walsh et al. (1996) described the use of heterodyne detectors by volunteers in a large-scale survey of bat activity in relation to land class in the United Kingdom. Heterodyne detectors have several drawbacks, one of the most important being their restricted bandwidth for detecting ultrasound (typically $\pm$ 5-8 kHz around the tuned frequency). Frequency division has been widely used in bat surveys,

especially in the United States where Anabat detectors are popular (e.g., Lance et al. 1996; Murray et al. 1999). We have used time expansion and direct sampling of ultrasound to survey bats in Europe. This is partly because we are interested in describing (and classifying) echolocation calls of bats with as little information loss as possible. Moreover, time expansion detectors are more sensitive than frequency division models (Fenton et al. 2001) partly because they use microphones that are more sensitive across a broader bandwidth. Time expansion detectors therefore detect more calls per unit time and presumably at greater distances (Fenton et al. 2001). However, because calls cannot be recorded while call sequences are being downloaded to recording media (typically 20 s for downloading a 10x expanded 2 s sequence of ultrasound), considerable sampling time is wasted when using time expansion.

A major advance in recording methods involves direct sampling of ultrasound to computer hard disks, whereby fast sampling PCMCIA (Personal Computer Memory Card International Association) data acquisition cards can be used to sample ultrasound without encountering aliasing problems (Pettersson 1999). Direct sampling allows minimal information loss from signals, high sensitivity and continuous recording for long time periods e.g.,, one hour with 16-bit resolution and a

2.4 Gb hard disk). Because direct sampling involves use of a laptop computer in the field, it is sometimes more practical to carry a small time expansion detector linked to a tape or DAT recorder. We will therefore describe how both time expansion and direct sampling methods can be used in surveys of bat activity but start by describing methods of acoustic identification. We will cover aspects of survey design, before finally describing results from some case studies of acoustic monitoring of bats in Europe.

Analysis: Discriminant Function Analysis and Neural Networks

The first stage in developing an objective method for acoustic surveys is to record and analyze echolocation calls to develop a call library from species in your study area. Bat echolocation calls exhibit considerable intraspecific variation because of the effects of acoustic clutter on call design (Schnitzler and Kalko 1998) and through inter-individual variation related to age, gender and morphology (reviewed in Jones et al. 2000). We argue that it is best to be conservative in assessing the degree of species identification by recording the study species in as many ecological circumstances as possible. This means recording bats in clutter, in open habitats, and even when exiting roosts. If acoustic surveys are based across a wide range of habitats, it

is important that call variation in relation to habitat be taken into consideration. Information on features such as distance of the bat to clutter, position of the microphone relative to the bat and so forth can be invaluable. It is also important to record a large number of individuals (not calls), as calls from individual bats should be used in statistical analyses to avoid pseudoreplication. In our studies we select one call per bat for analysis (Parsons and Jones 2000; Vaughan et al. 1997a).

Once a call library is available, it is possible to assess the reliability of acoustic identification to species. Temporal (e.g., pulse duration, pulse interval) and frequency (e.g., highest and lowest frequency, frequency of most energy) parameters are extracted from calls for multivariate analysis. It is important to realize that frequency divided output may not give an accurate measure of call duration (see Fenton et al. 2001).

Techniques such as discriminant function analysis (DFA: e.g., Krusik and Neefus 1996; Lance et al. 1996; Vaughan et al. 1997b; Zingg 1990) and neural networks (Parsons and Jones 2000) can be used to classify calls made by different species according to multivariate analysis of call parameters. Neural networks may achieve even higher rates of correct classification to species than DFA (Parsons and Jones 2000). The most

problematic species to discriminate acoustically in temperate regions are often bats in the genus *Myotis*, many of which produce brief, broadband frequency-modulated (FM) calls. Nevertheless, some *Myotis* species can be identified with confidence, often through differences in starting and end frequency, and bandwidth (Parsons and Jones 2000). Using time-expanded calls, Vaughan et al. (1997) correctly classified 67% of calls from four *Myotis* species and *Plecotus auritus* by using DFA (random classification would have been 20% correct). Parsons and Jones (2000) were able to identify 82% of 5 *Myotis* species correctly by using artificial neural networks, with classification rates for individual species varying between 75% (*M. daubentonii*) and 90% (*M. nattereri*).

Multivariate methods have been used successfully for species identification from time-expanded calls even in bat communities where species richness is high. The analyses of Vaughan et al. (1997) and Jones and Parsons (2000) dealt with 13 and 12 species respectively in the United Kingdom. Russo and Jones (2002) recently applied DFA to calls from 18 Italian species, and obtained a correct classification rate of 82%. In developing methods for acoustic identification, typically species that can be identified unambiguously from call structure are removed (e.g., several rhinolophid species, and the low frequency - ca. 11 kHz

- echolocator *Tadarida teniotis* in Italy), and restrict the DFA to species with similar calls. Always check that the assumptions of DFA analyses are met (sometimes quadratic, rather than linear analyses must be used), use cross validation, and consider specifying prior probabilities (sample sizes).

The manner in which output from multivariate analysis is handled must be considered. A cutoff degree of certainty in identification can be specified so that calls, which are not classified with a specified degree of confidence, are regarded as 'unclassified'. Having a known degree of confidence in certainty of identification is worthwhile, and some researchers may wish to limit analyses to calls that lie in areas of multivariate space where identification is unambiguous. We prefer not to do this, because call designs associated with particular habitat features (especially clutter) may have to be removed from the analysis. Consequently, the number of times that a particular species is scored as being present in habitats where identification is not absolute would be underestimated. Indeed, the effect of habitat features (e.g., foliage) on detectability of echolocation calls requires further research.

Survey Design

Active versus remote monitoring

We prefer active monitoring for two reasons. First, equipment for recording time-expanded echolocation calls is expensive, so the purchase of several units necessary for most remote monitoring studies is often not feasible. Second, active monitoring maximizes encounter rates with bats, whereas a remote unit placed atone site may repeatedly record the same individual animal. We therefore walk transects through habitat patches. Bat detectors are used in frequency division mode; so all frequencies used by bats in our study areas can be detected. When a bat pass is heard, time-expanded sequences are recorded to tape for species identification. Vaughan et al. (1997) recorded in stereo from two detectors, one set to time expansion, the other to frequency division.

Two 1 km transects were walked for 45 mins at a fixed walking speed, starting 30 mins after sunset. Each transect was confined to one land use type. Meteorological data were recorded, and transects were not walked in heavy rain because of the risk of equipment damage.

Surveys may also combine active and static monitoring, whereby a series of different sites are monitored for a fixed time each over one night. We adopted this approach in our study of bat activity on organic versus conventional farms (see below). It is also worth considering whether transect features (e.g.,

presence of a footpath through woodland) are likely to bias the chances of encountering bats.

Replication and randomization

Vaughan et al. (1997) recorded bat activity in 10 different land use types. Three replicates of each were studied, with a minimum distance of 5 km between replicates. Each site was visited 3 times, once before the main period of lactation, once during lactation, and once after most bats had finished lactating. With multiple visits to the same transect in different seasons, transects were walked in the same direction so that order effects were standardized. Sites were visited in random order within each block of 10 land use types in each season. This approach allowed analysis using ANCOVA (analysis of covariance), after transformation of bat pass data to achieve normality. Site was nested within land use type, season was a crossed factor, and temperature was a covariate. This is effectively a repeated-measures analysis of variance (ANOVA), with each season considered as a repeat.

Russo and Jones (2003) used a similar approach in a study of bat activity in relation to habitat type (10 categories) in southern Italy, but increased the number of replicates for each habitat type to 6, and visited each site once only. Future studies could incorporate species accumulation curves to determine the optimum number of transects walked in each habitat (see Walsh

et al., this volume). In both of our studies (Russo and Jones 2003; Vaughan et al. 1997), we used post-hoc tests (Bryant-Paulson Tukey tests) on adjusted means (effects independent of the covariate) to determine which habitats differed from one another in terms of bat activity. Activity was measured by monitoring the numbers of bat passes. An index of feeding activity relative to searching for prey was calculated as the ratio of feeding buzzes to bat passes.

Paired sampling

If 2 habitats or situations are being compared, paired sampling is a powerful technique because it controls for variation in bat activity due to environmental factors. Vaughan et al. (1996) used paired sampling (in this case while recording frequency divided calls) to investigate the effects of water quality on bat activity. In this study, sewage output was used as a surrogate measure of water quality. Two people sampled for bats at sites upstream and downstream of sewage outputs simultaneously. Sites upstream and downstream from nineteen separate sewage outputs were sampled to achieve statistical power, and Wilcoxon signed rank tests were used to test whether the difference between the number of passes upstream and downstream differed significantly from zero (Fig. 1). Higher activity occurred upstream in 14 of 19

pairs, and significantly more bat activity occurred overall upstream compared with downstream.

Currently, paired sampling is being used to determine the effects of agricultural intensification on bat activity, testing the hypothesis that bat activity is higher on organic farms than on conventional farms (Wickramasinghe et al. submitted). If agricultural intensification has had a detrimental effect on bats, we predict that activity will be higher on organic farms where many methods of intensification (hedgerow removal, use of pesticides and artificial fertilizers) are absent.

The project involves direct sampling of ultrasound (after detection by frequency division) at sample points within habitats using a paired site design. Detailed habitat surveys are conducted to match pairs of farms that are as similar as possible to one another, with the exception of farm management, one farm using conventional farming methods and the other being farmed using organic methods (as defined by The Soil Association, UK, see http://www.soilassociation.org/sa/saweb.nsf/standards/index.html). Paired farms of similar sizes were no more than 5 km apart, which controlled for geographic variation. Four comparable habitats were selected for sampling at each farm pair (pasture, arable, water,

woodland), with each habitat type being extensive enough for 3 sampling points at least 15 m from each other. The order of habitats sampled within a pair was the same, but visitation of habitat types between pairs was randomized. Both sites within a pair were sampled on consecutive nights and recording commenced 1 hour after sunset. Although simultaneous sampling of sites would control for night-to-night variation, the nature of the equipment being used made this impossible. Sites within a pair were therefore matched for weather conditions, and temperature differences had to be within 4 degrees of when the first site was sampled. Other environmental variables e.g., wind speed, and habitat structure variables were measured at each sample point and included in the analysis.

Paired sampling methods can still be undertaken even if there are more than 2 treatments to compare by employing repeated measures ANOVAs. Paired sampling and repeated measures designs increase statistical power compared with unpaired designs by separating variability among treatments from variability among replicates. Ninety-five percent confidence intervals are thus reduced, making it easier to detect differences between treatments.

Case Studies

Acoustic surveys of habitat use by British bats revealed that once total bat activity was adjusted for air temperature, activity (of all species combined) was significantly higher over rivers and lakes than over other land use types investigated (Fig. 2: Vaughan et al. 1997). Especially interesting was a difference in habitat use by 2 recently described cryptic species of pipistrelle, discovered by analyzing differences in echolocation calls (Jones and van Parijs 1993). The '45 kHz phonic type' (*Pipistrellus pipistrellus;* Jones and Barratt 1999) was a generalist in habitat use. Although this species was detected most frequently over rivers and lakes, activity was also common in most other habitats surveyed (Fig. 3a). Conversely, the '55 kHz phonic type' (*P. pygmaeus;* Jones and Barratt 1999) concentrated its activity near lakes and rivers (Fig. 3b). These patterns of habitat use are consistent with dietary studies, which suggest that *P. pygmaeus* feeds on insects with aquatic larvae more than *P. pipistrellus* (Barlow 1997).

Habitat surveys of a similar design to those of Vaughan et al. (1997) were conducted in Mediterranean habitats in southern Italy (Russo and Jones 2003), in a landscape affected by human activity for over 300 generations (Blondel and Aronson 1999). Bat activity

was most frequently recorded over rivers and lakes (Fig. 4), confirming that these habitats are important bat foraging areas over a wide geographic scale.

Given that riparian habitats are important over a wide geographic area (see also Racey 1998), is it possible to identify particular landscape features along rivers that are especially important for bats? Warren et al. (2000) used time expansion detectors to investigate the distribution of *M. daubentonii* and *P. pipistrellus* in relation to small-scale variation in riverine habitat. Eight 1.5 km transects were walked at constant speed along a 13 km stretch of river, starting 30 mins after sunset. Both species were most active along stretches of the river with smooth water surfaces and with trees on both banks. Smooth water surfaces facilitate the detection of prey on water surfaces by echolocation (important for *M. daubentonii*), and stretches of river with trees on both banks had more insects associated with them than stretches without trees (Warren et al. 2000; Fig. 5).

Conclusions

We have illustrated some of the ways in which time expansion and direct sampling can be used in studies of habitat use by bats. Our focus has been on riparian habitats. We show that rivers and lakes support higher levels of bat activity than other habitats studied in

Britain and in Italy. Water quality can affect bat activity, and tree lines can increase the value of riparian habitats for bats.

Time expansion and direct sampling are the only methods suitable for accurate descriptions of acoustic parameters of bat echolocation calls (Fenton 2000; Fenton et al. 2001). However, time expansion detectors and direct sampling of ultrasound have been used in relatively few surveys of habitat use by bats. More research has been conducted with heterodyne and frequency division equipment. The expense of time-expansion detectors and the loss of recording time during downloading of call sequences are 2 drawbacks of this methodology. However, bat detectors linked to time expansion devices record more bats per unit time of recording than do some frequency division detectors (Fenton 2000; Fenton et al. 2001). The high sensitivity and relatively flat frequency response of some of the microphones supplied with the best time expansion detectors are important specifications for scientists interested in recording sounds from species that emit calls of low intensity and/or high frequency. Direct sampling overcomes problems of lost recording time. The increased information content of calls recorded by time expansion and direct sampling is also likely to result in better discrimination of species with similar call structure. Preliminary results suggest that *Myotis*

species may be discriminated more confidently using time-expanded calls than by frequency-divided recordings, for example (L.P. Wickramasinghe, unpub. data). Therefore direct sampling, with its advantages of high sensitivity, and retention of maximal information content of calls offers great benefits for future studies of habitat use by bats. In choosing bat detectors, trade offs between recording quality and cost are inevitable (Fenton 2000), and the high quality offered by the recording techniques described in this paper may not be necessary for all studies of habitat use, although they are essential for describing the echolocation calls of bats (Fenton et al. 2001).

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FIGURE LEGENDS

FIG. 1. Results from a study of bat activity using paired sampling. Differences in log bat passes represented as values upstream minus downstream of sewage outlets at 19 sewage treatment works. Both measures show higher upstream values (see Vaughan et al. 1996).

FIG. 2. Surveys of bat activity in relation to land use type in England indicate that bat activity is highest over rivers and lakes. Bars are adjusted mean log transformed counts of bat passes recorded in 10 land use types (Rivers (Ri), Lakes (La), Unimproved grassland (Ug), Amenity grassland (Ag), Improved cattle pasture (Ip), Arable land (Al), Villages (Vi), Ancient semi-natural woodland (Aw), Conifer plantations (Cp), Mixed plantations (Mp) -

habitats defined in Vaughan et al. (1997a)). Groups of habitat types supporting activity levels, which are not significantly different from one another are indicated by the same letter. Bars represent the means of nine transects (three sites visited three times each) with standard deviations shown.

FIG. 3. Two cryptic species of pipistrelle exhibit different patterns of habitat use. Bars are adjusted mean log transformed counts of passes for the 55 kHz phonic type of *P. pipistrellus* (suggested name *P. pipistrellus*)(a) and for the 45 kHz phonic type (suggested name *P. pygmaeus*) (b). Abbreviations and conventions as in Fig. 1. Figure from Vaughan et al. 1997a.

FIG. 4. Median and interquartile range of bat passes recorded (all species) in 10 habitats in southern Italy. Habitats are Lakes (La), Rivers (Ri), Beech woodlands (Bw), Chestnut woodlands (Cw), Mediterranean macchia (Mm), Arable land (Al), Rural towns (Rt), olive groves (Og), Mediterranean and sub-Mediterranean woodlands (Mw) and Conifer plantations (Cp).

FIG. 5. (a) *Pipistrellus pipistrellus* has highest activity along stretches of river, which are smooth and have trees on both banks. (b) Highest insect densities are recorded along river stretches that are smooth and tree-lined. Means ± SDs are illustrated. Figure from Warren et al. (2000).

FIG. 1.

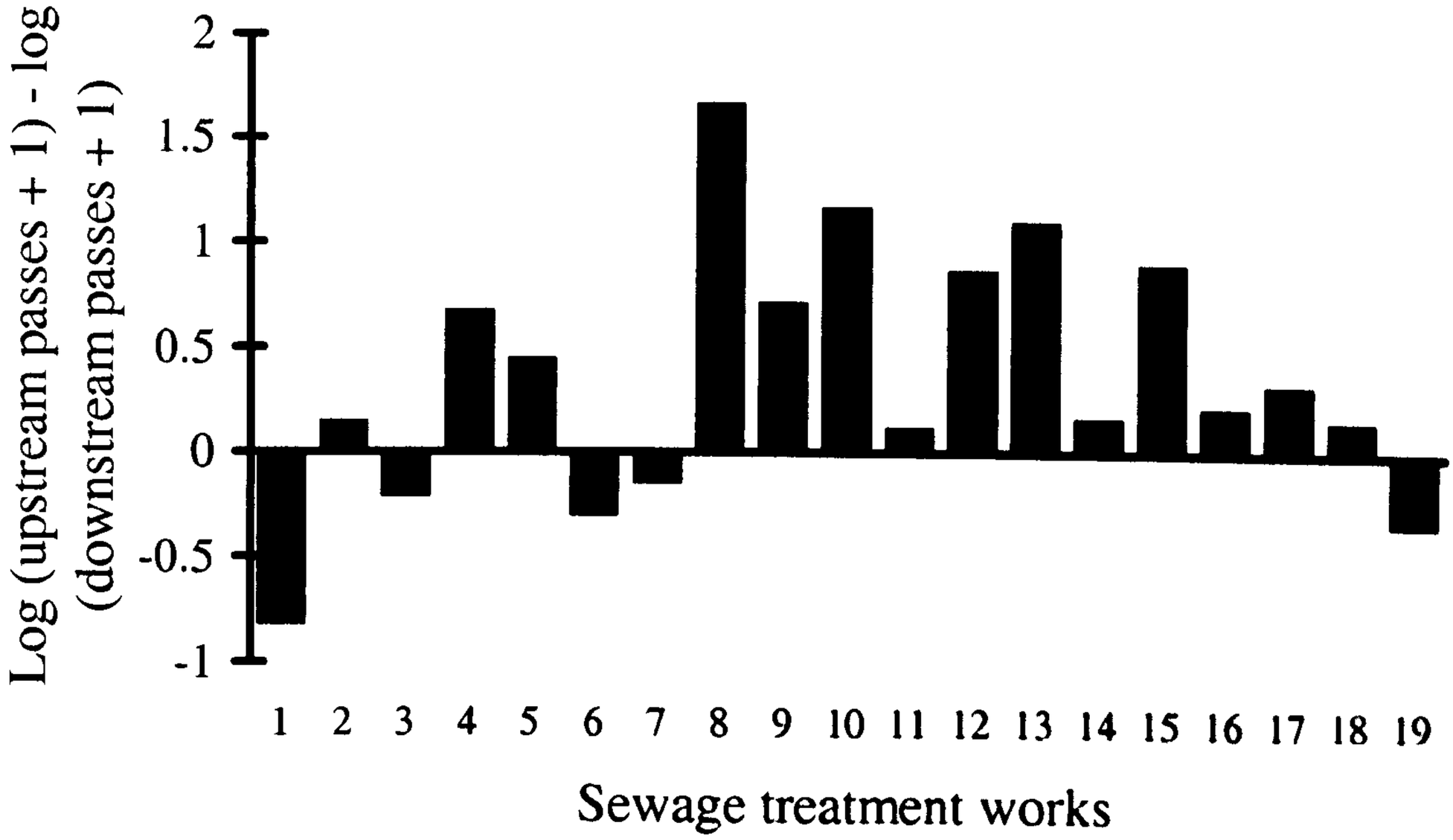

Log (upstream passes + 1) - log (downstream passes + 1)
2
1.5
1
0.5
0
-0.5
-1
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Sewage treatment works

FIG. 2.

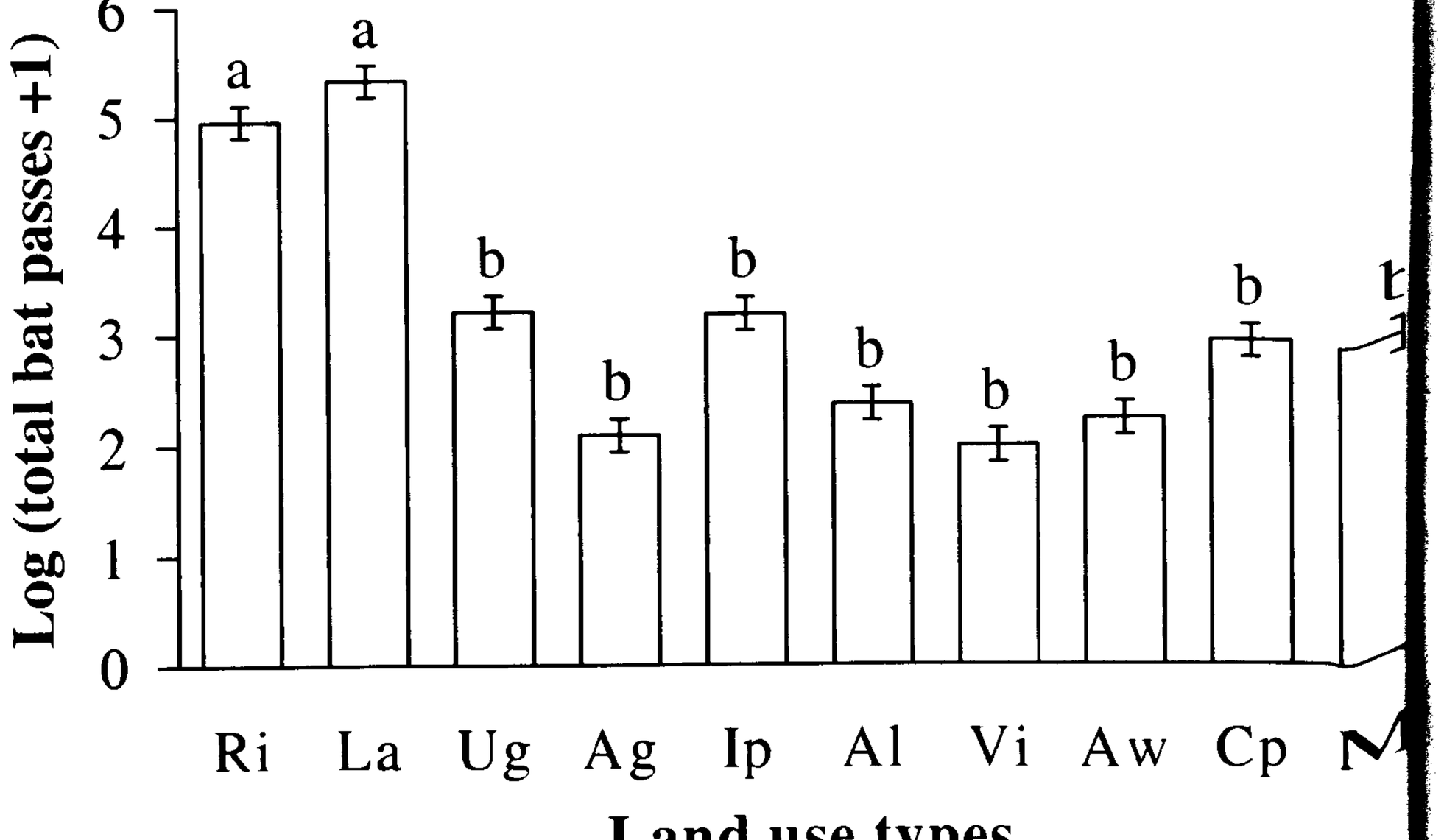

Log (total bat passes +1)
0
1
2
3
4
5
6
a
a
b
b
b
b
b
b
b
Ri
La
Ug
Ag
Ip
Al
Vi
Aw
Cp
Land use types

FIG. 3.

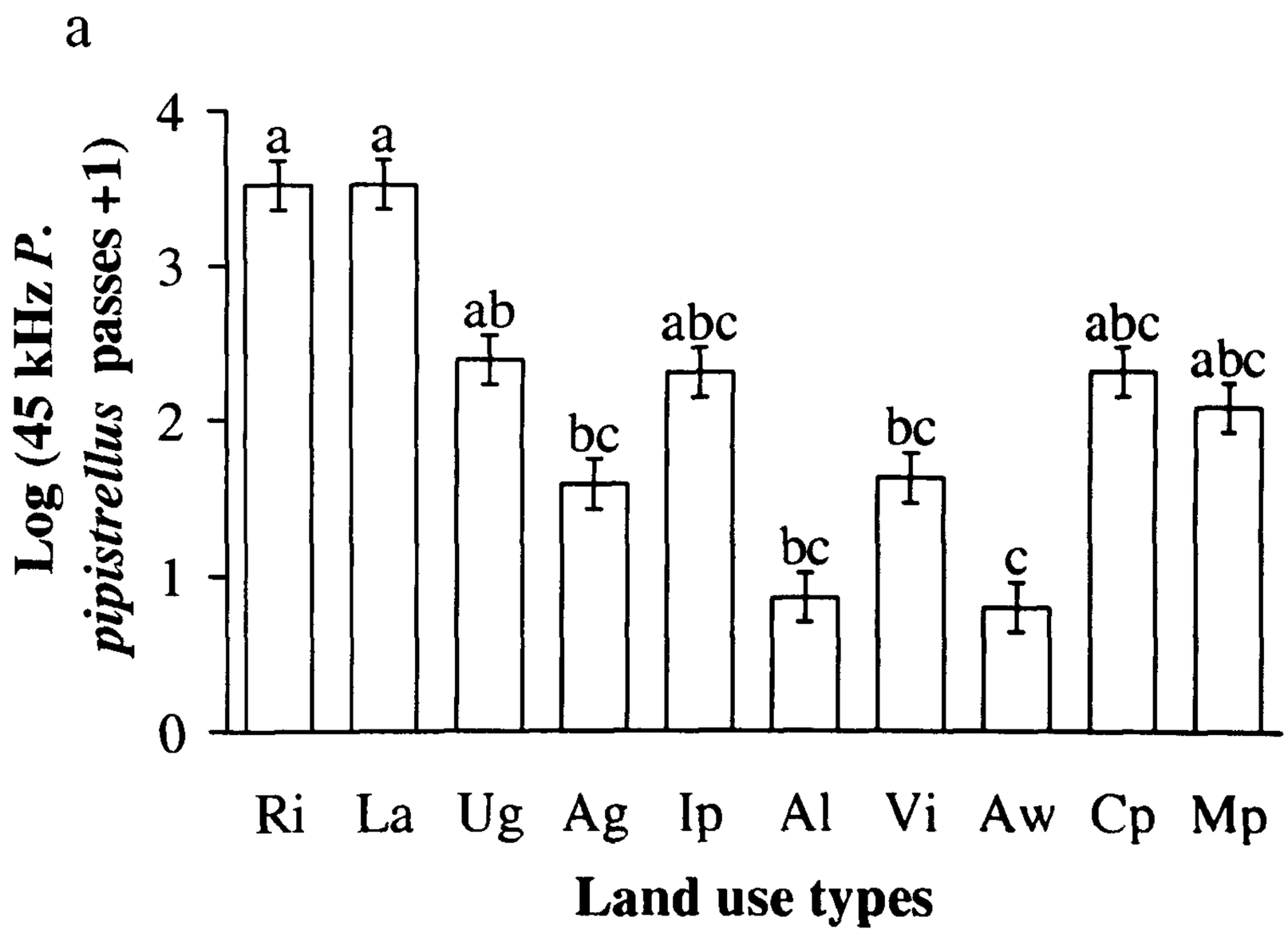

a
Log (45 kHz P. pipistrellus passes +1)
0
1
2
3
4
a
a
ab
bc
abc
bc
bc
abc
abc
Ri La Ug Ag Ip Al Vi Aw Cp Mp
Land use types
b
Log (55 kHz P. pipistrellus passes +1)
0
1
2
3
4
5
a
a
b
b
b
b
b
b
b
b
Ri La Ug Ag Ip Al Vi Aw Cp Mp
Land use types

FIG. 4

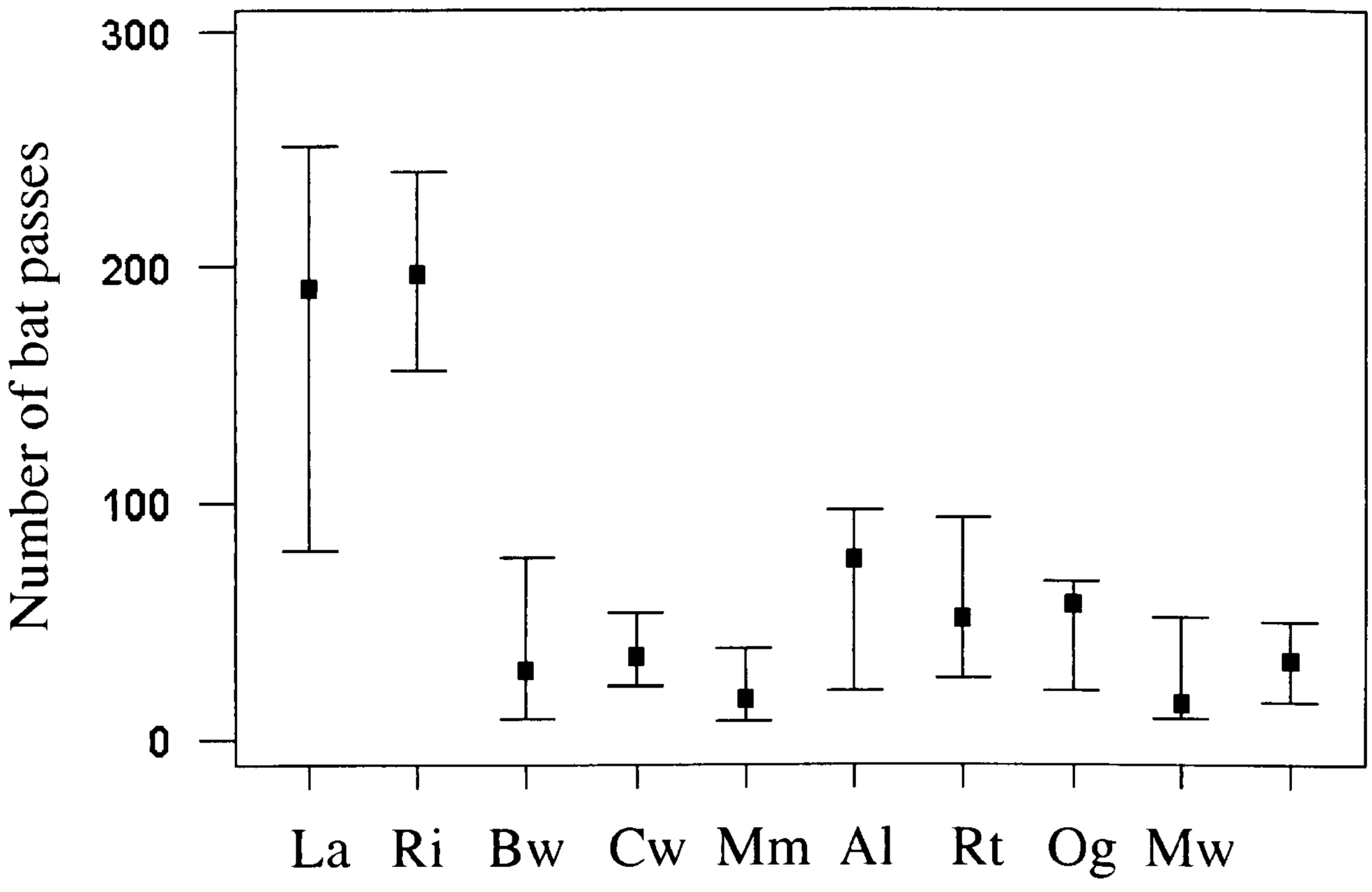
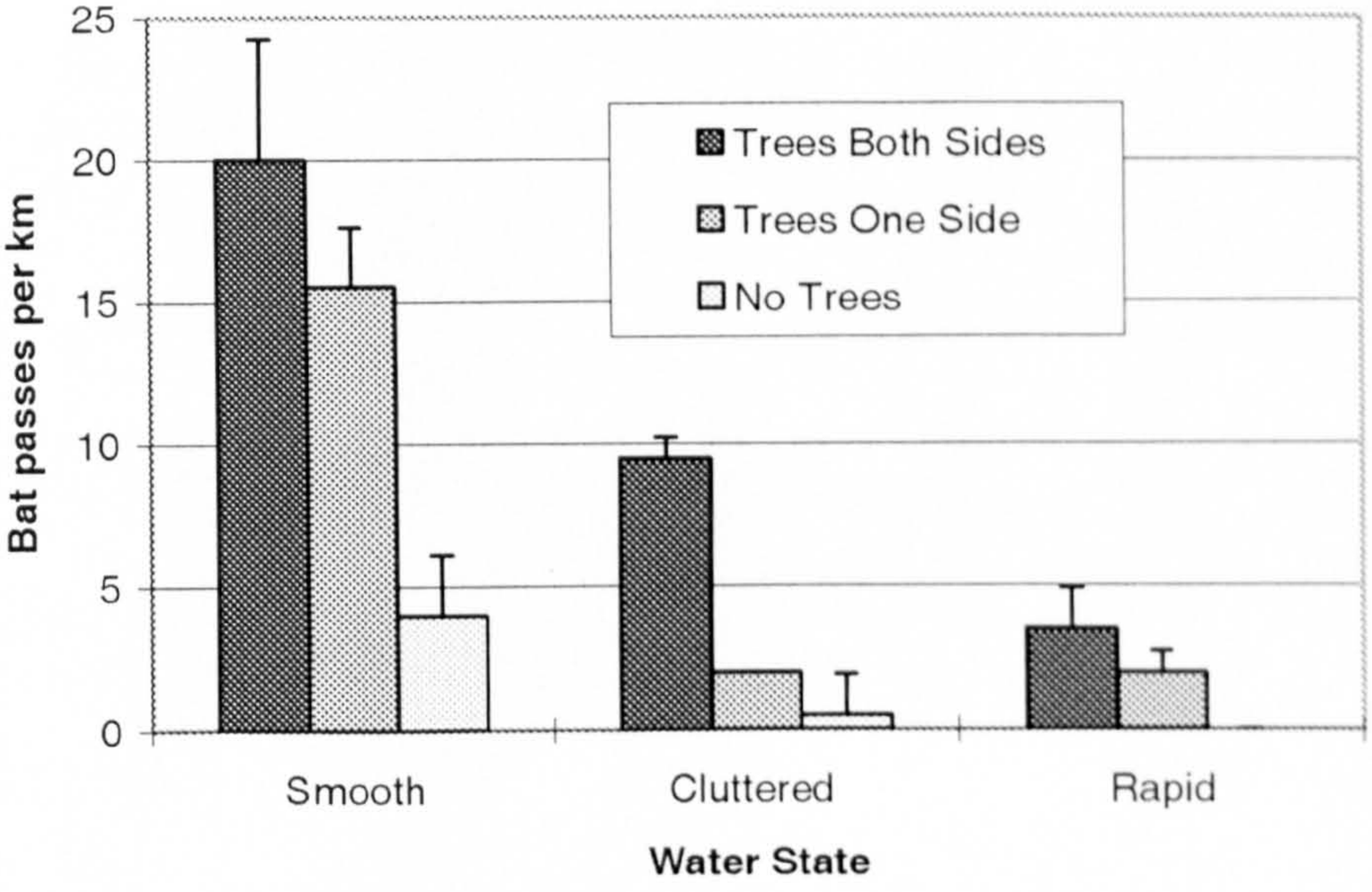


FIG. 5

a



b